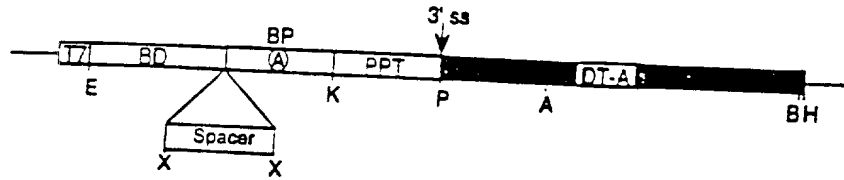


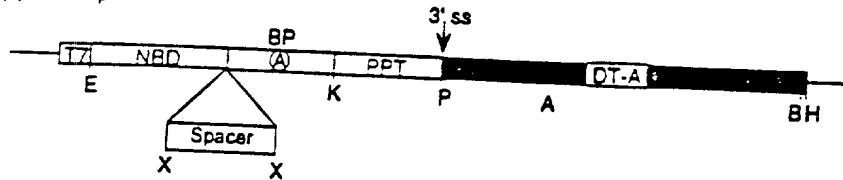
# FIGURE 1A



(B) (1) pPTM+Sp



(2) pPTM-Sp



(C)

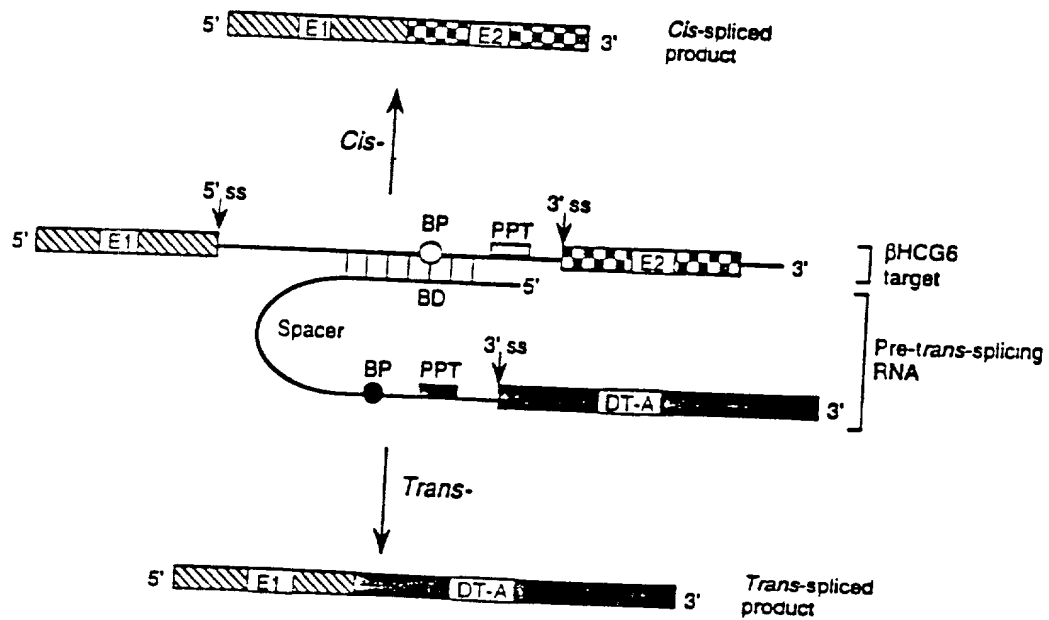
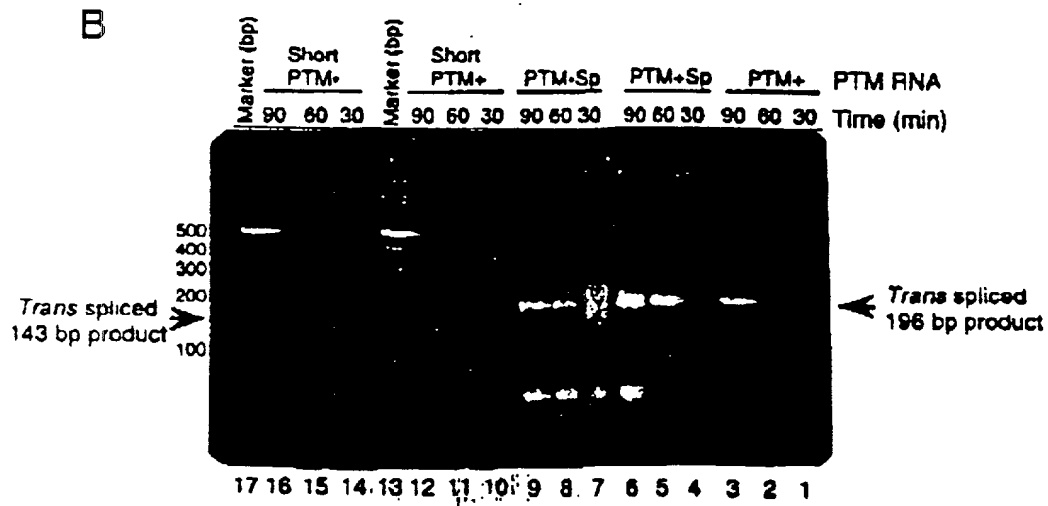
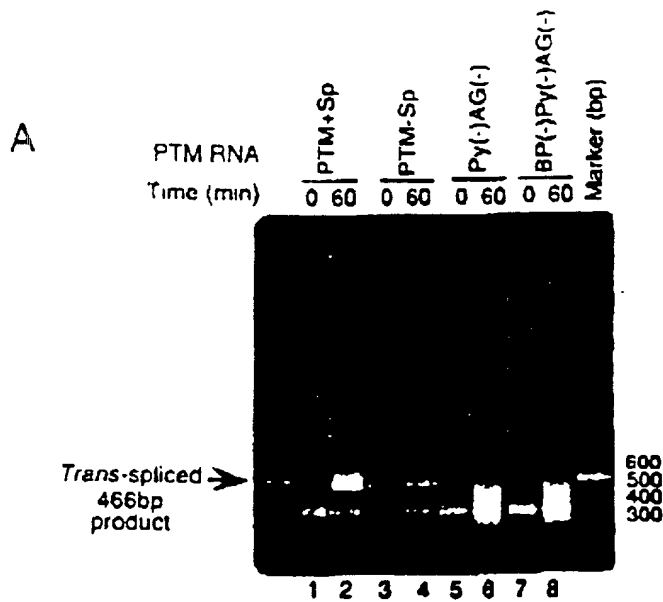
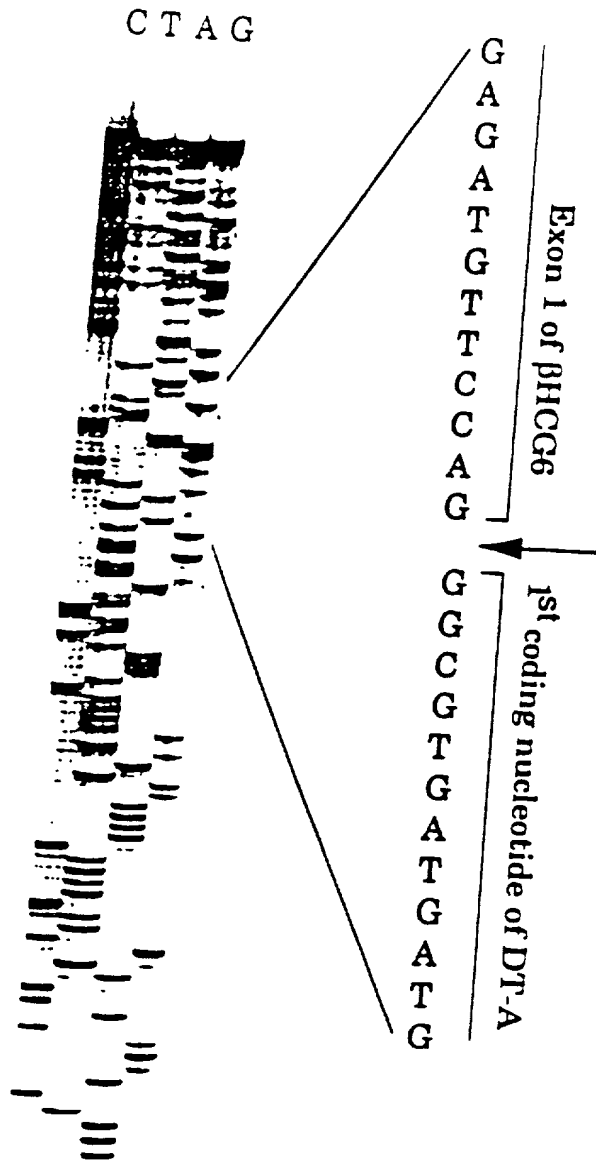


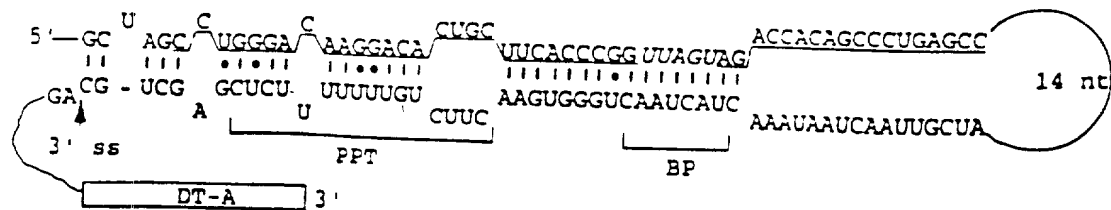
Figure 1 B-C



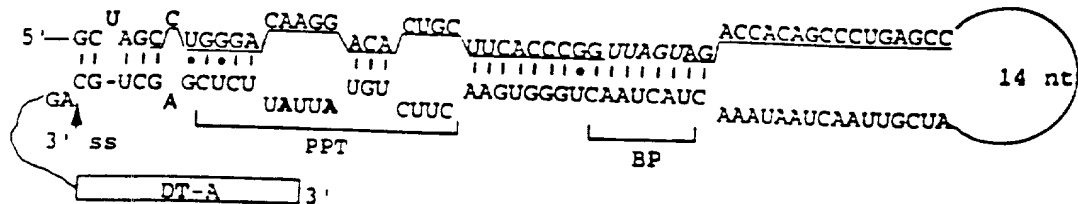


(A)

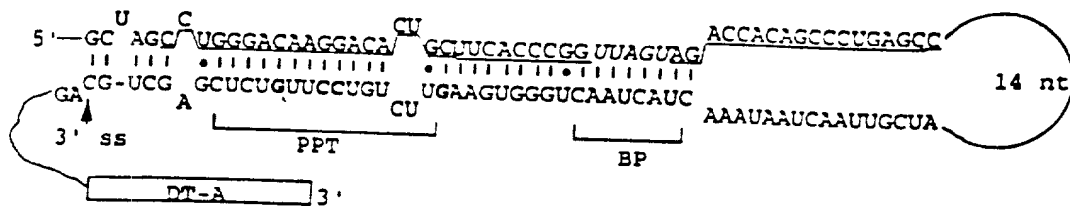
1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:



(B)

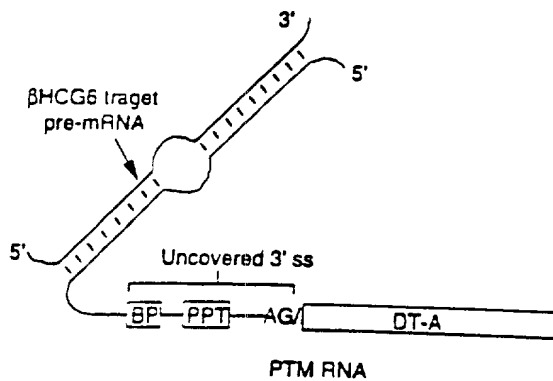


Figure 4A-B

(C)

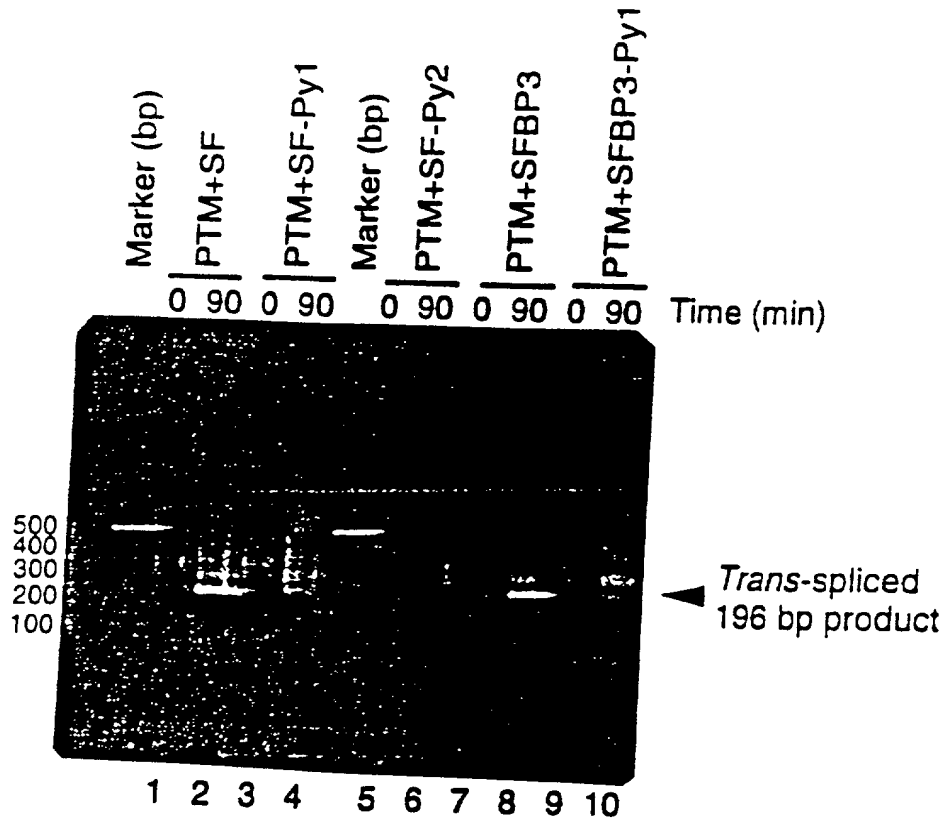


Figure 4c

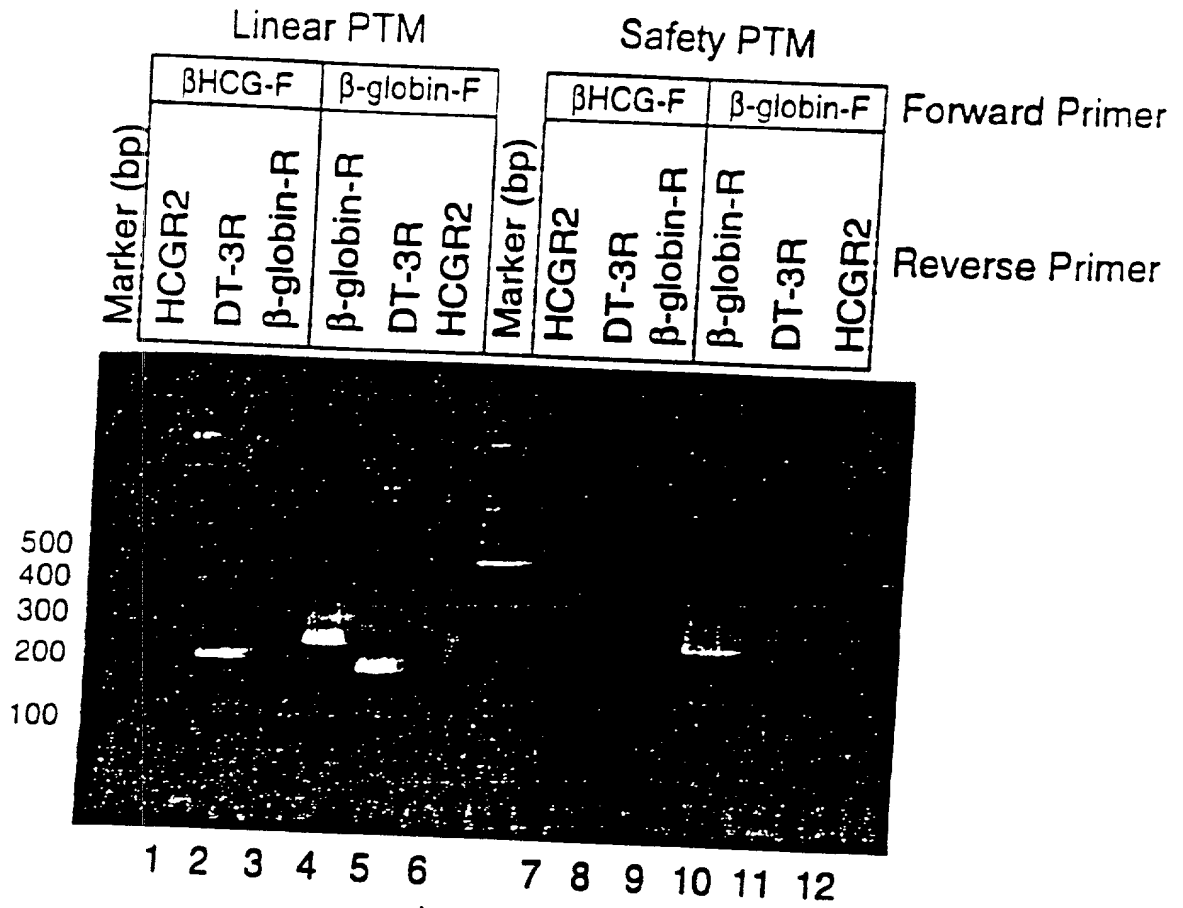


Figure 5

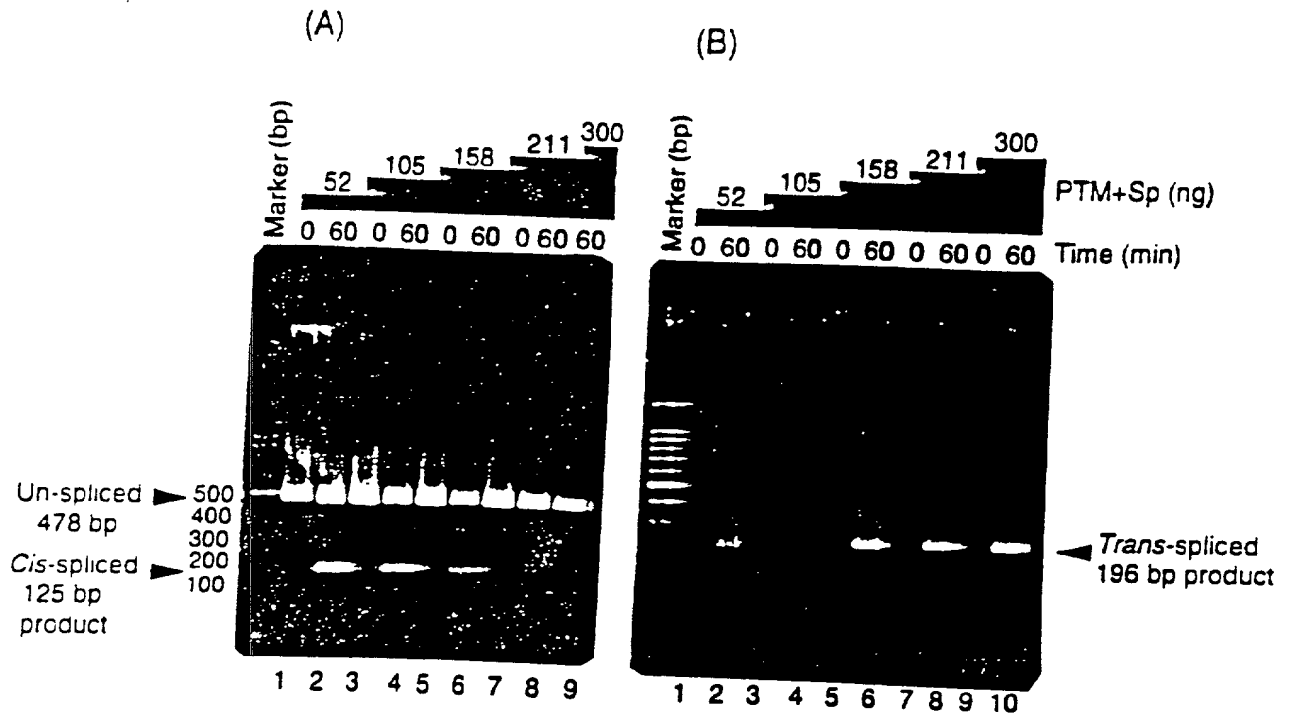
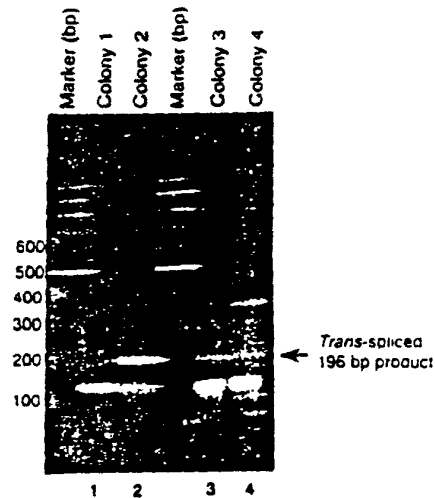


Figure 6



Figure 7

(A)



(B)

Exon 1 of  $\beta$ HCG6 ↓  
 5'-CAGGGACCGCACCAAGGATGGAGATGTTCCAG-GGCGCTGATGATGTTOTT  
 ↑ 1st coding nucleotide of DT-A  
 GATTCTTCTTAAATCTTTTGTGATGGAAAACCTTTCTTCGTACCACGGGACTA  
 AACCTGGTTATGTAGATTCCATTCAAAA-3'

# Double Splicing Pre-therapeutic RNA

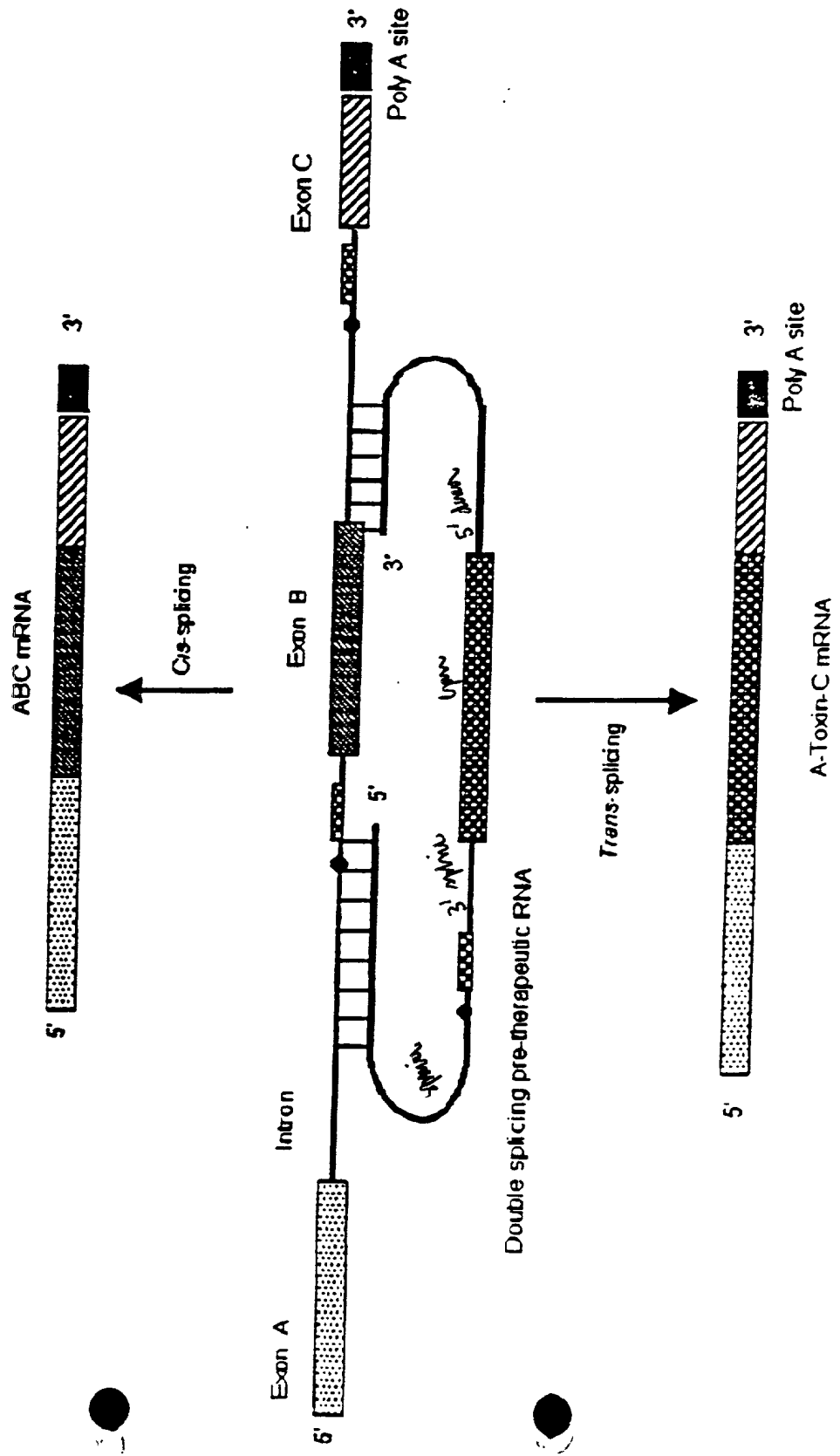
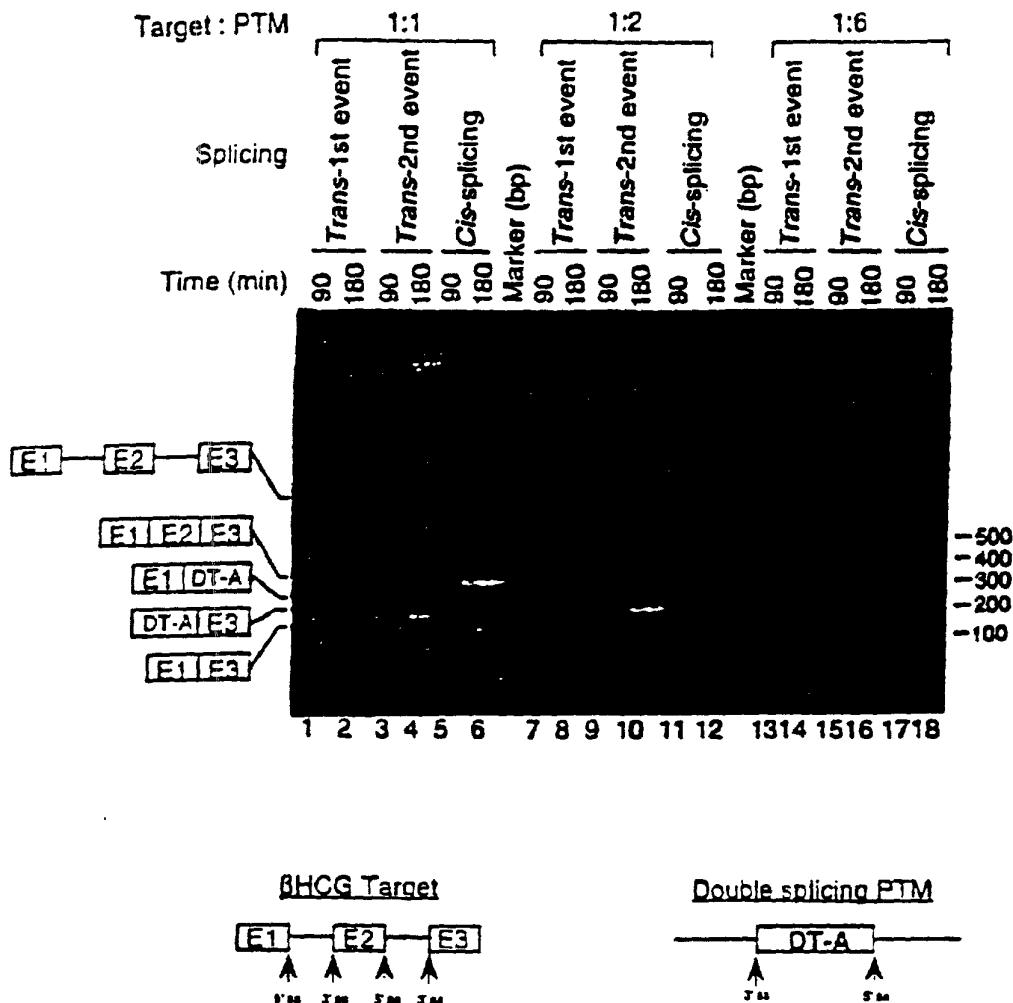


Figure 8A

## Selective Trans-splicing of a Double Splicing PTM

(3' ss of PTM to 5' ss target and, 5' ss of PTM to 3' ss of target)



### Cis-spliced products

**E1-E2-E3** = Normal cis-splicing (277bp)

**E1-E3** = Exon skipping (110bp)

### Trans-spliced products

**E1-DT-A** = 1st event, 196bp. Trans-splicing between 5' ss of target & 3' ss of PTM.

**DT-A-E3** = 2nd event, 161bp. Trans-splicing between 3' ss of target & 5' ss of PTM.

Figure 8B

31304B-A  
(Sheet || Of 58)

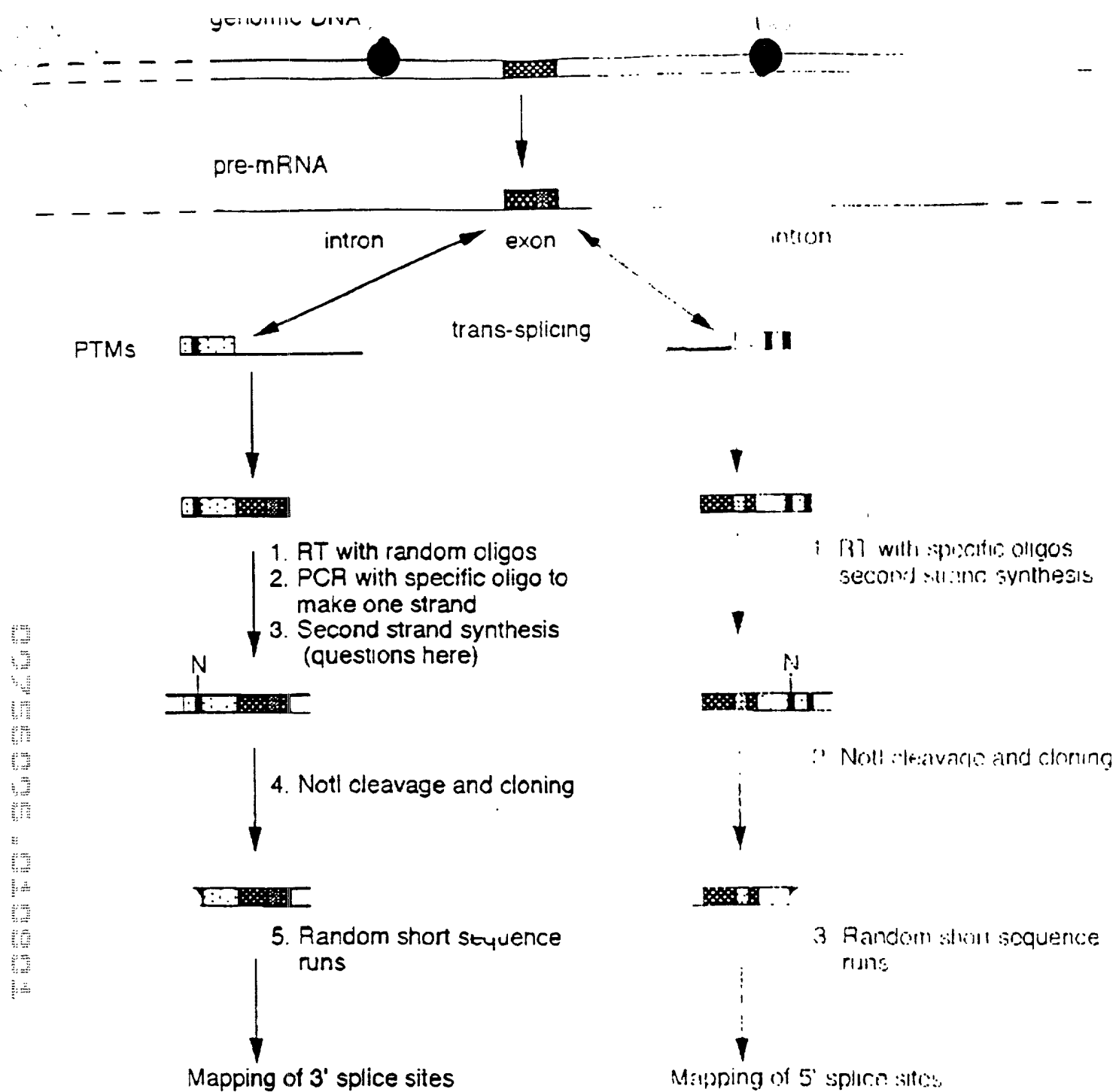
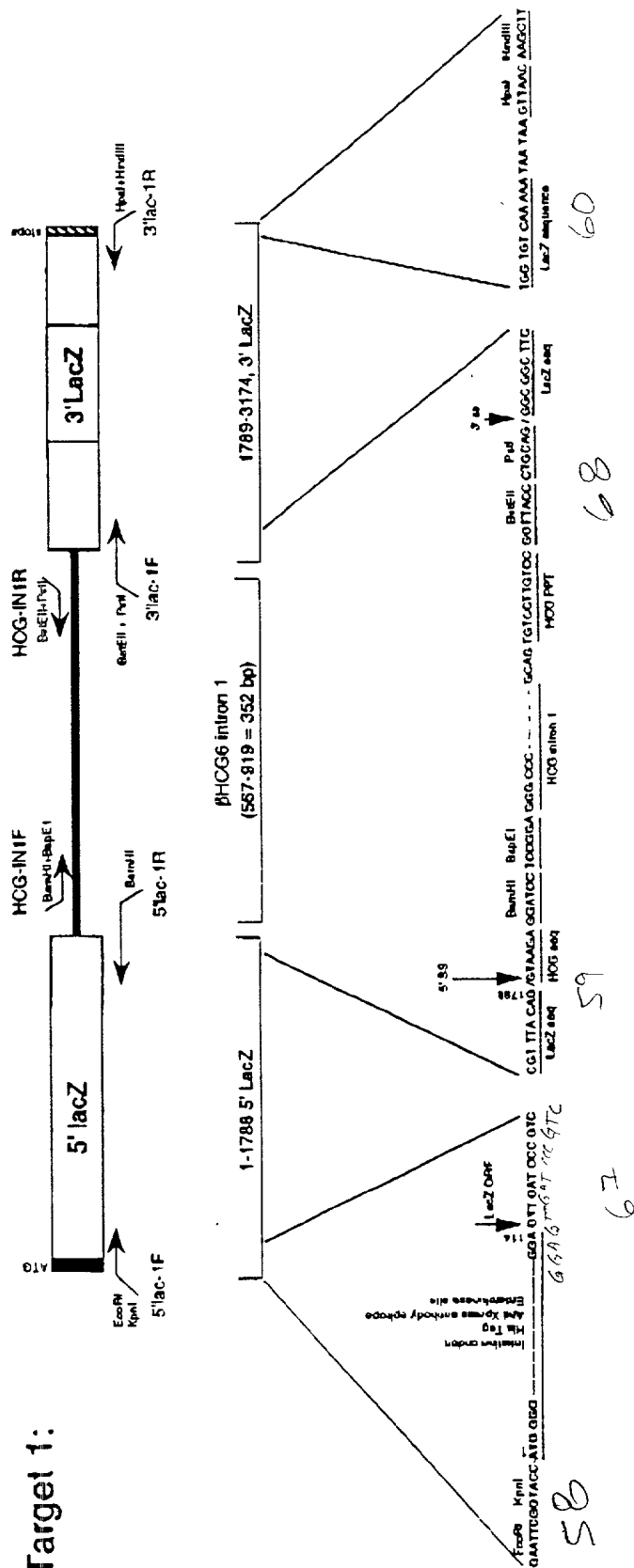


FIGURE 9

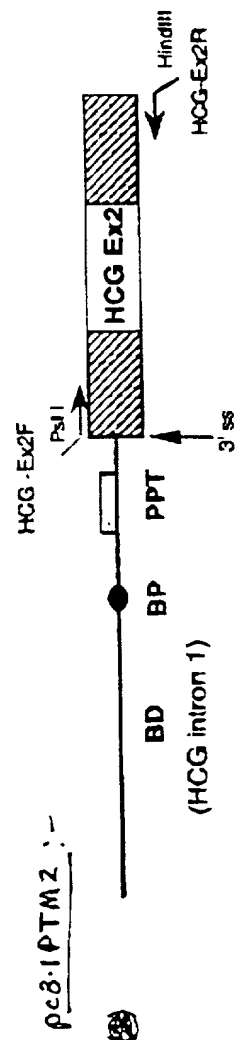
31304B-A  
(Sheet 12 Of 58)

# Knack Out

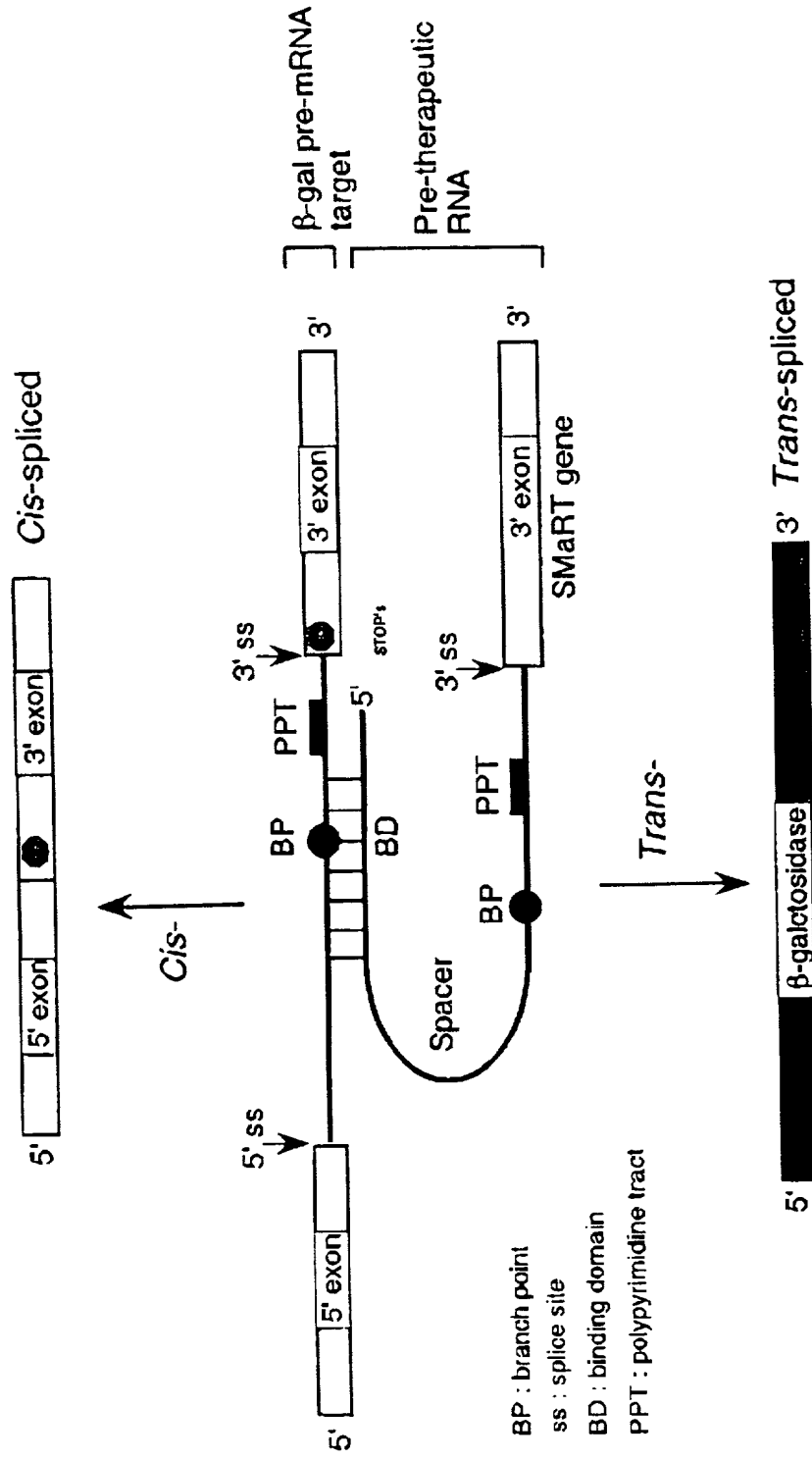
**Target 1:**



PTMS



# Restoration of $\beta$ -Gal activity by SMaRT (Spliceosome Mediated RNA *Trans*-splicing)



31304 B-A  
(Sheet 15 of 58)

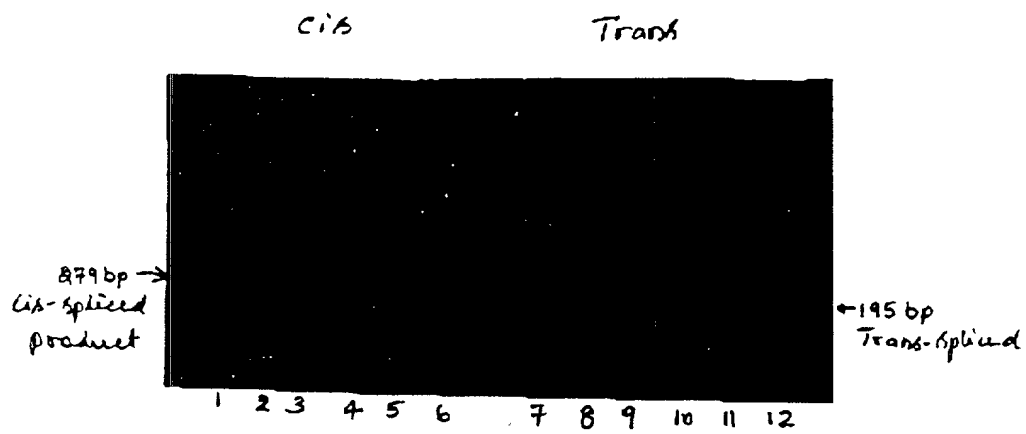


FIGURE 11A

31304 B-A  
(Sheet 16 of 58)

Figure 11 B



51504 15-11  
(Sheet 17 of 58)

FIGURE 11C

# Nucleotide Sequence Demonstrating that *Trans*-splicing is Accurate

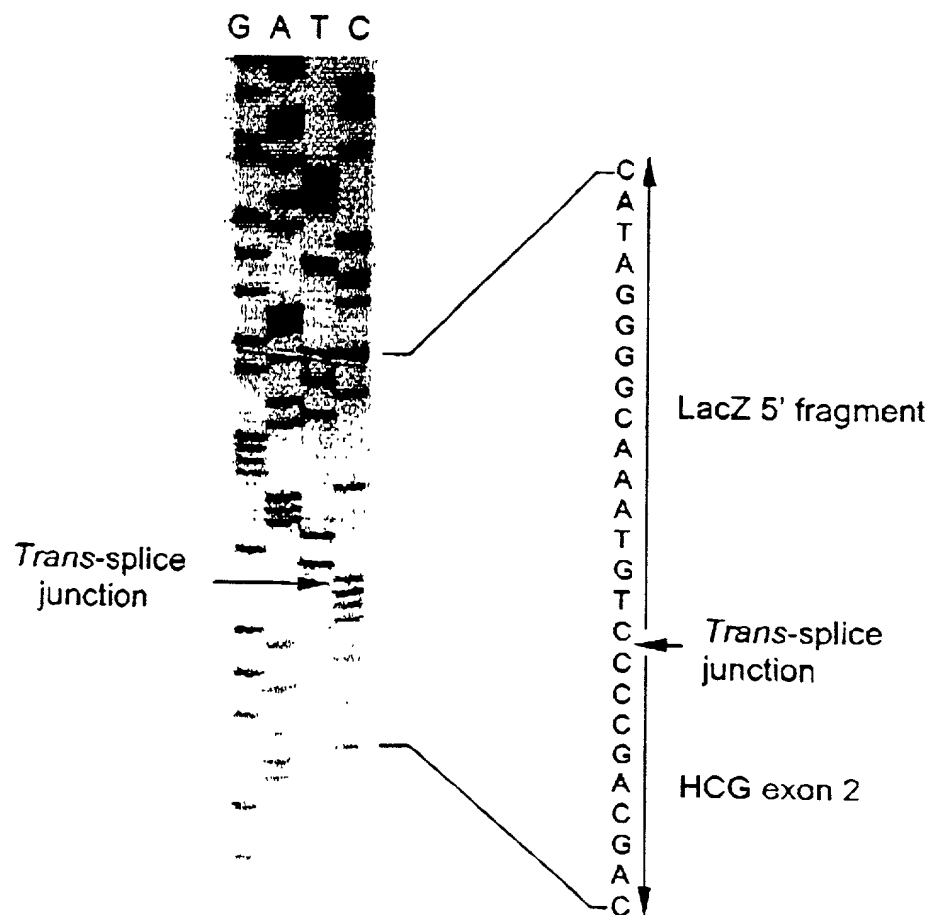


FIGURE 12 A

31304-B-A  
(Sheet 18 of 58)

(1). Nucleotide sequences of the cis-spliced product (285 bp) :

BioLac-TR1

GGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAAACAGTCTTG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/CGCGGCTTCGTC~~TA~~ATAATG

GGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACGGCAACCCGTCGGTCGCTTACGGCGGTGATT

Lac-TR2

TGGCGATACGCCGAACGATCGCCAGTTCTGTATGAACGGTCTGGTCTTTGGCGAC~~CG~~CACGCCGCATCCAG

(2) Nucleotide sequences of the trans-spliced product (195 bp)

BioLac-TR1

GGCTTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAAACAGTCTTGG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAG/GGGCTGCTGCTGTTGCTGCTGCT

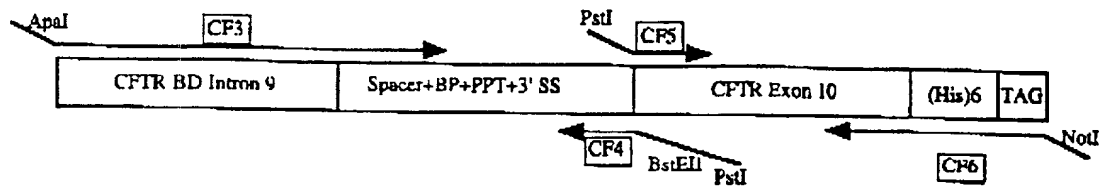
HCGR2

GAGCATGGGCGGGACATGGGCATCCAAGGAGCCACTTCGGCCACGGTGCCG

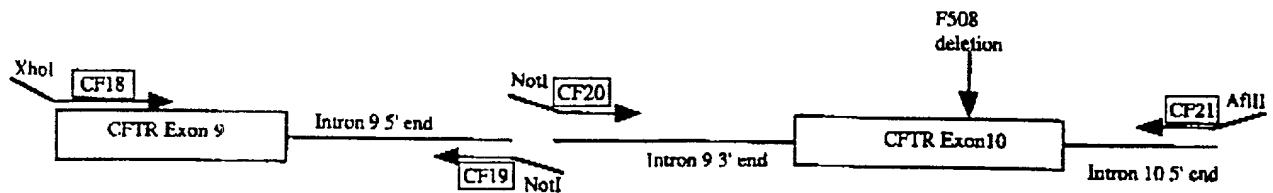
Figure 12 B

31304-B-A  
(Shut 19 of 58)

# CFTR Pre-therapeutic molecule (PTM or "bullet")



## CFTR mini-gene target - Construction



## TRANS-SPLICING Repair

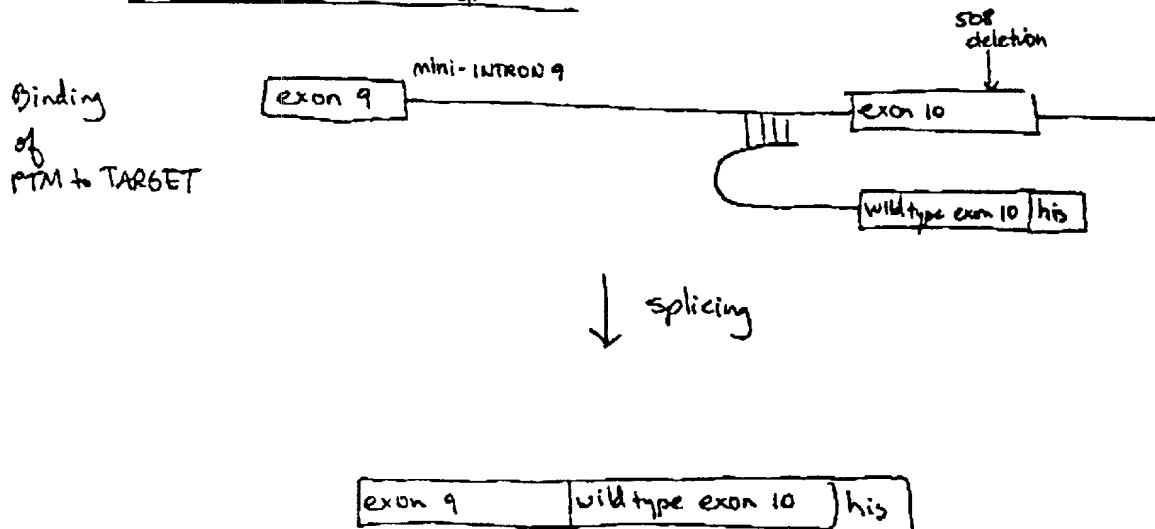
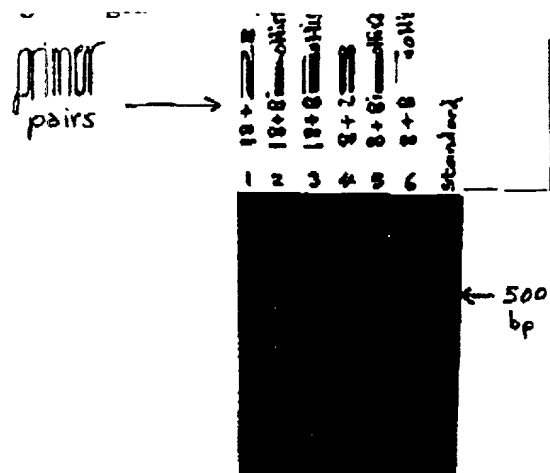


Figure 13

31304-B-A  
(shut 2004.58)

Figure 14

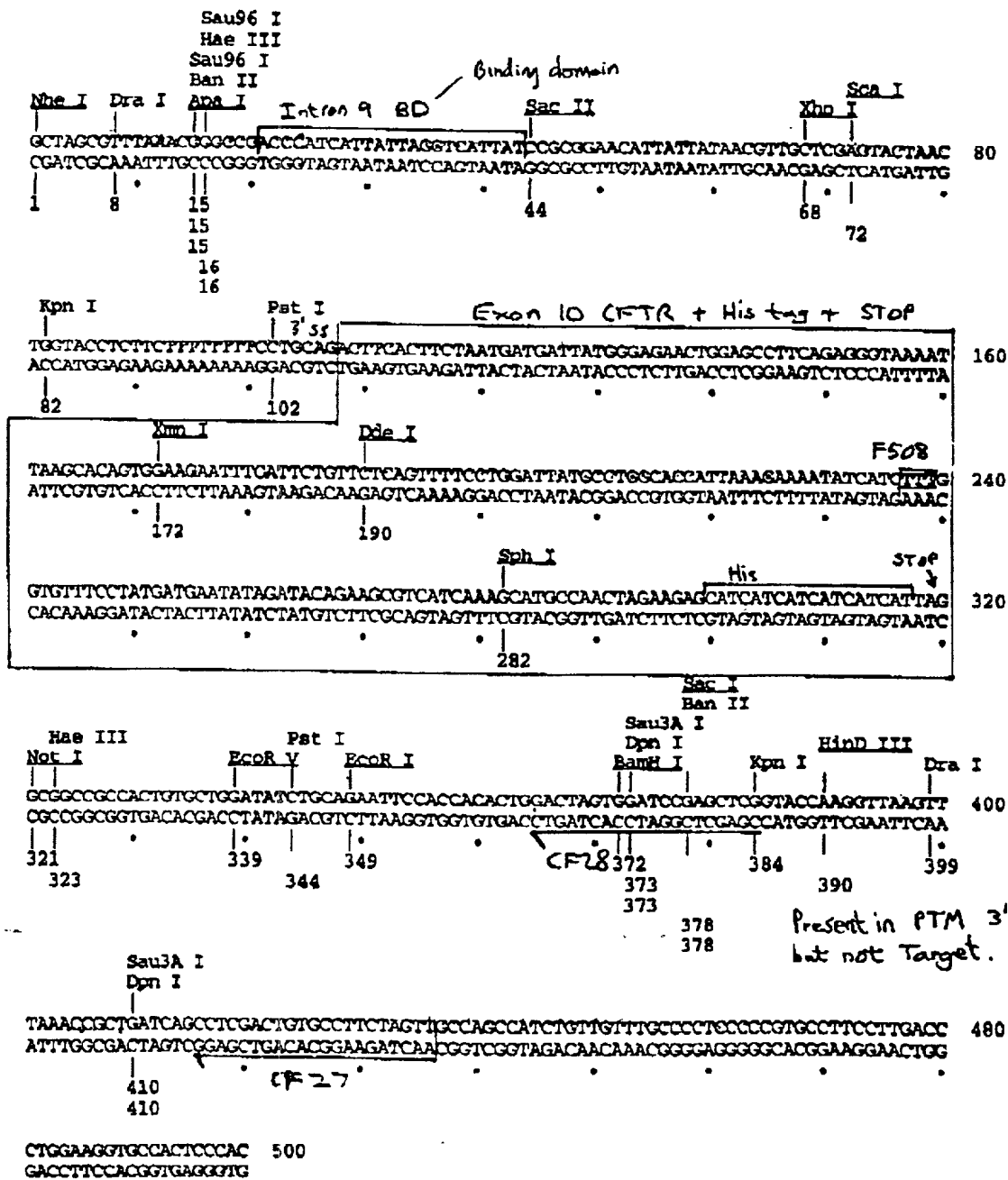


31304 B-A  
(Sheet 21 of 58)

# FIGURE 15

DNA sequence 500 b.p. GCTAGCGTTTAA ... TGCCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)



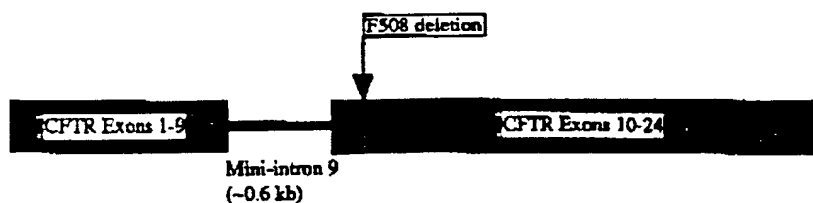
31304-A-B  
(Aunt 22 of 58)

**EXPERIMENT 2**

Repair of an exogenously supplied CFTR target molecule carrying an F508 deletion in exon 10.

PTM

+

CFTR Target  
(mini-gene)

Cotransfect PTM and Target molecules in HEK 293 cells  
and detect repaired CFTR mRNA by RT-PCR.

Repaired  
CFTR mRNA

Figure 1b

31304-A-B

Sheet 23 of 58

**EXPERIMENT 3**

Repair of endogenous CFTR  
transcripts by exon 10 invasion  
using a double splicing PTM

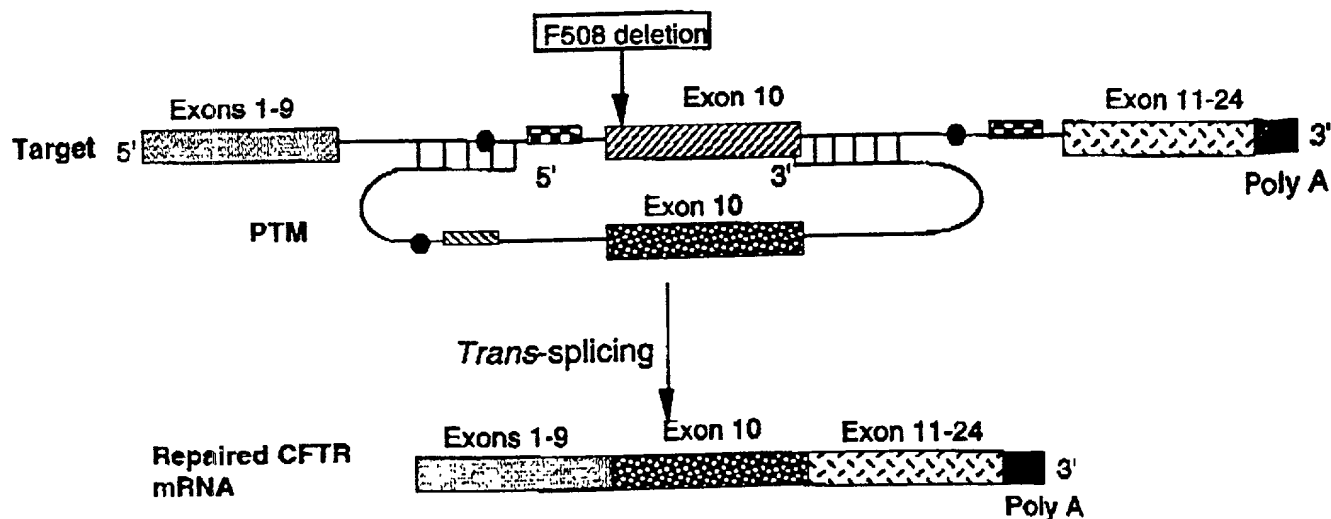
Double Splicing  
PTM

Figure 17

31304 B-A

Sheet 24 of 58



Sheet 25 of 58

## Double Trans-splicing Specific Target

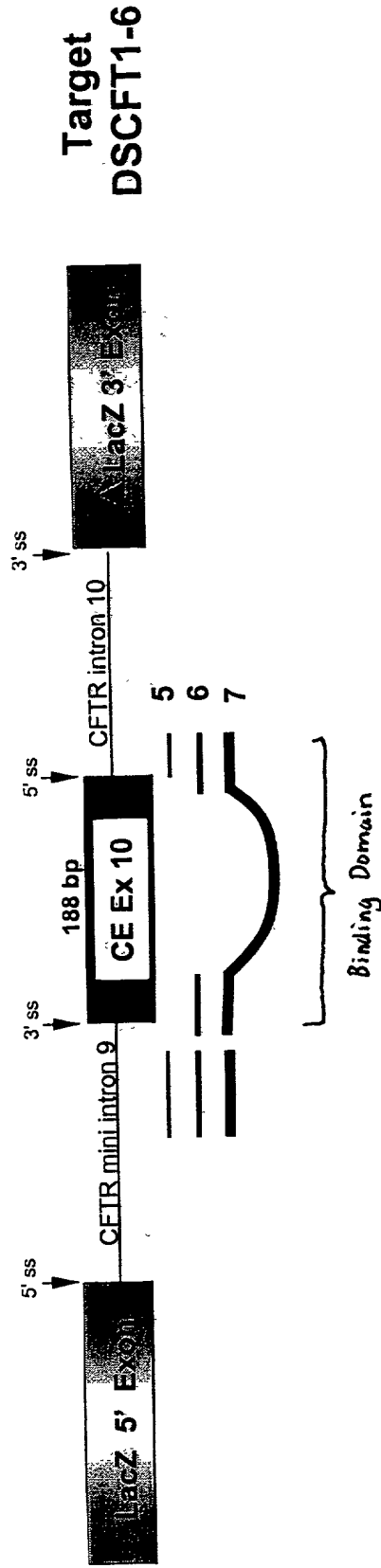
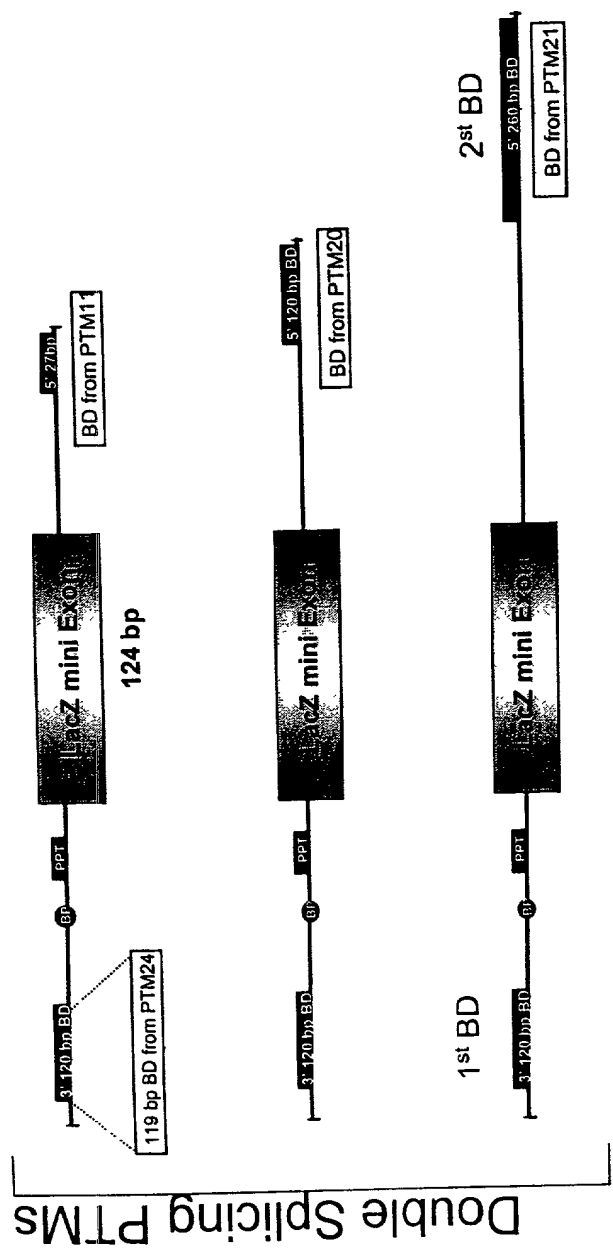


Figure 18

# Double Trans-splicing PTMs



- DSPTM-5**  
PTM with 27 bp BD & masks 5' single splice site
- DSPTM-6**  
PTM with 120 bp BD & masks both 5' & 3' splice sites
- DSPTM-7**  
PTM with 260 bp BD masking both the ss & the entire CFTR Ex10

Figure 19

Sheet 27 of 58

# Double Trans-splicing $\beta$ -Gal Model

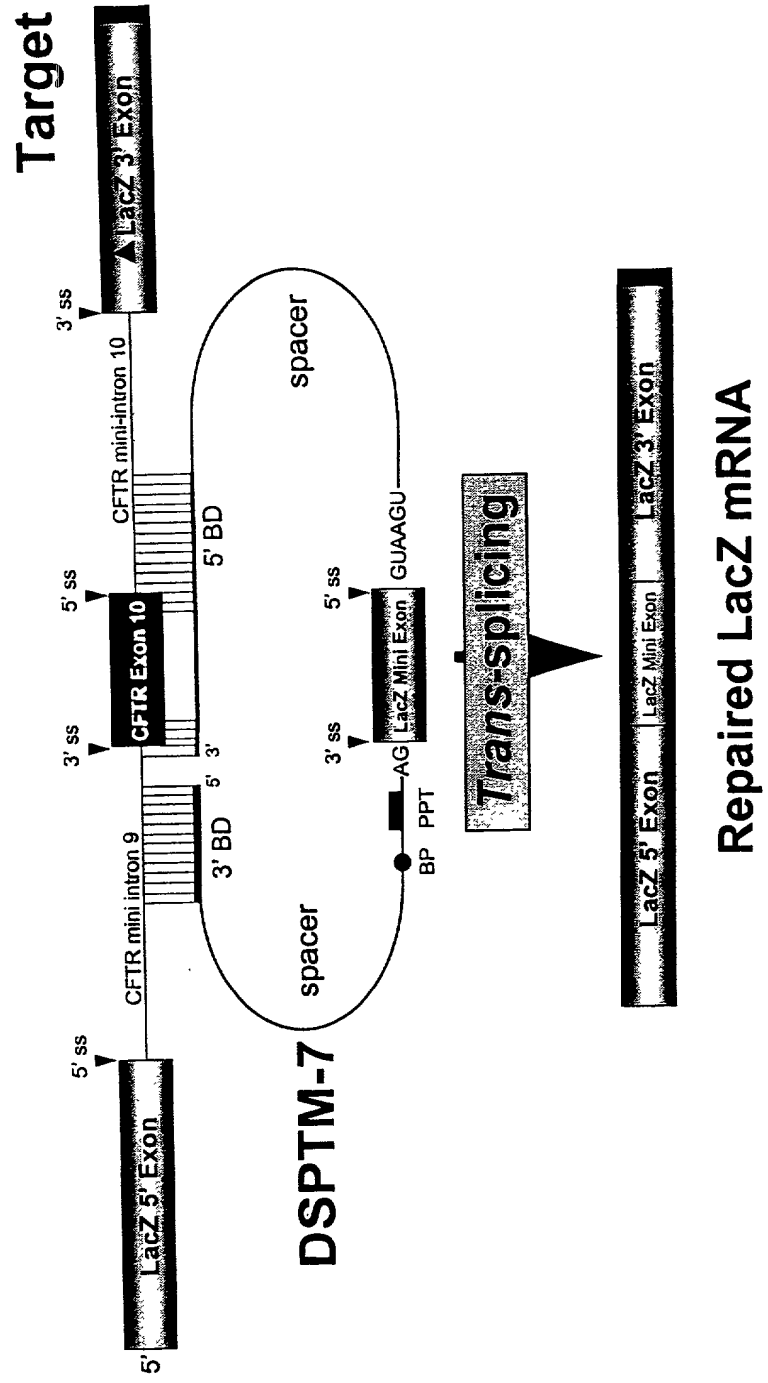
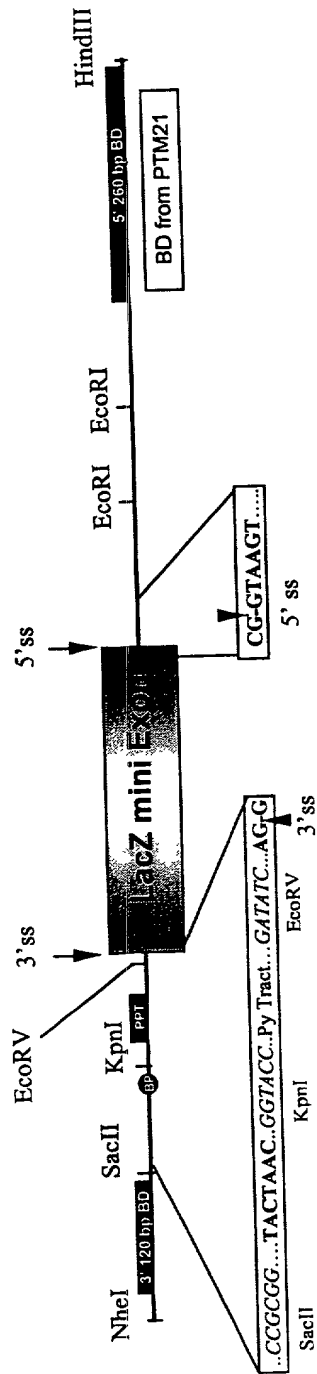


Figure 20

# Important Structural Elements of DSPTM-7: (Double splicing PTM with all the necessary splice elements i.e. has both 3' and 5' functional splice sites and the binding domains)



(1) 3' BD (120 BP) : GATTCACCTTGCTCCAAATTATCATCCTAAGCAGAGTGATATCTTATTGTAAAGATTCTATTAACTCATTTGATTTC  
AAAATATTTAAATACCTTCCTGTTTCATACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTGCTCGAA

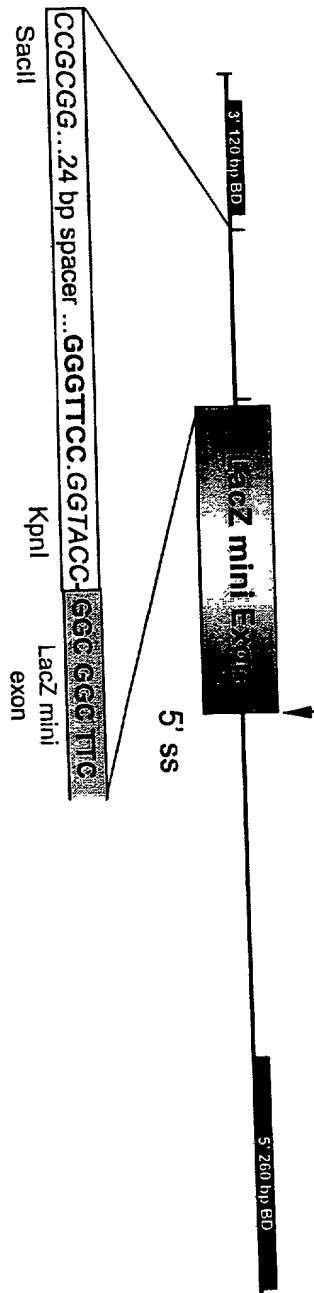
(3) Branch point, pyrimidine tract and acceptor splice site: TACTAAC T GGTAAC TCTTCTTTTTTTTTT GATATC CTGCAG GGC GGC  
BP Kpn I PPT EcoRV LacZ mini exon

(4) 5' donor site and 2<sup>nd</sup> spacer sequence: TCA ACG GTAAGT GTTATCACCGATATGTGTCTAACCTGATTCGGCCTTCGATACG  
5'ss LacZ mini exon  
CTAAGATCCACCGG

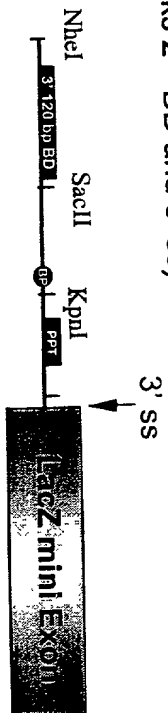
(5) 5' BD (260 BP) : TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAATTCATGCGCTTGTATCTATATTCATCATTTGGAA  
ACACCAATGATTTTCTTTAATGGTGCCTGGCATAATCCTGGAAACTGATAACACAAATGAAATTCCTCCACTGTGCTTAA  
AAAAACCCTCTGAATTCCTCCATTCTCCCATATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATTTAACTCA  
TTATCAAATCACGG

Figure 21

**DSPTM8** : (▲ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



**PTM29** (lacks 2<sup>nd</sup> BD and 5' ss)



**PTM30** (lacks 1<sup>st</sup> BD and 3' ss)

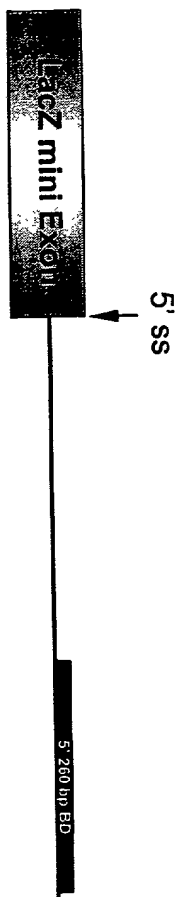


Figure 22

Mutants

### Accuracy of Double *Trans*-splicing Reaction

Sheet 30 of 58

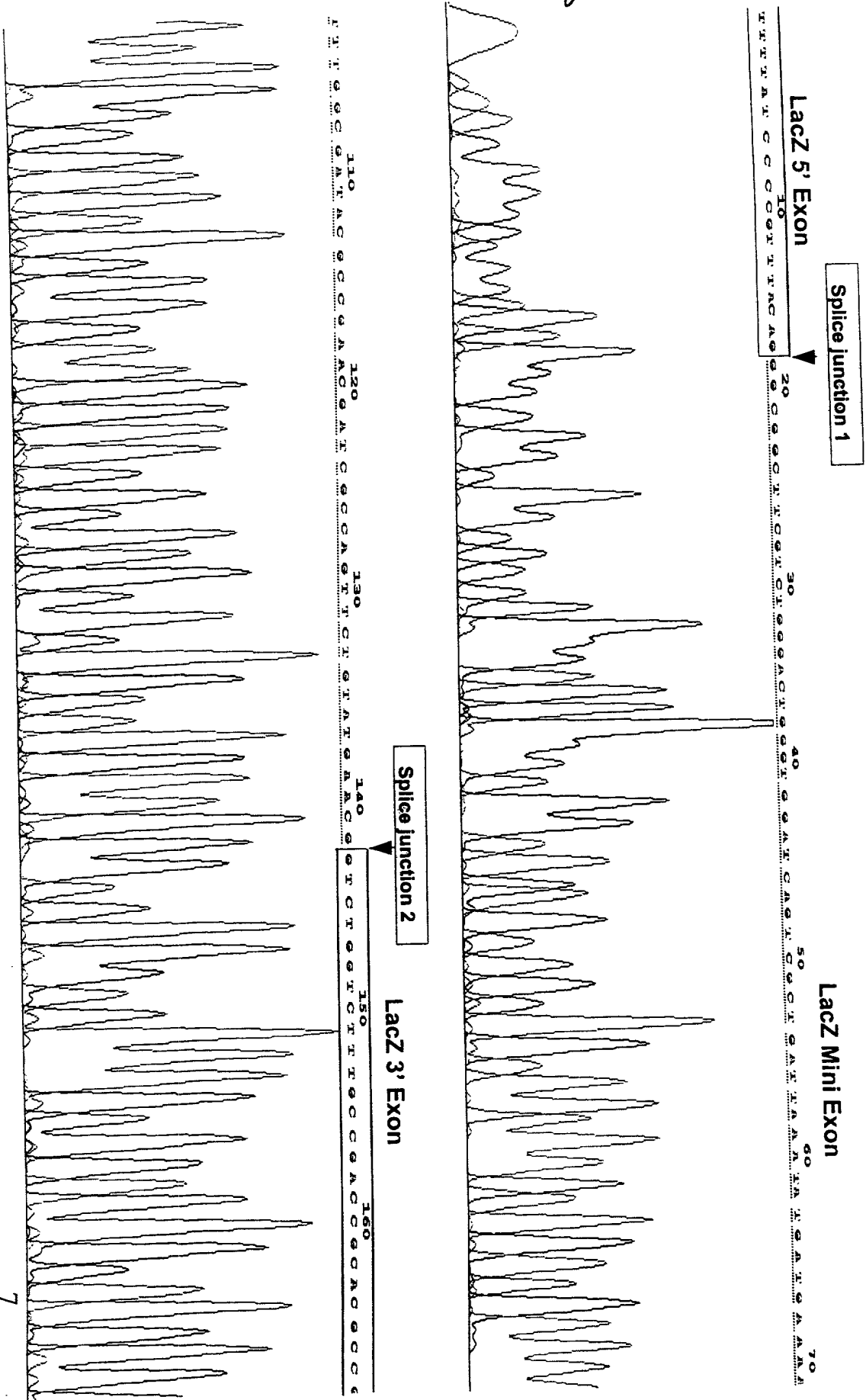


Figure 23

about 31 of 58



Lane 1: DSCFT1.6 Target alone	25 µg
Lane 2: DSPTM7	25 µg
Lane 3 Target + PTM #6	25 µg
Lane 4: Target + PTM #9	25 µg
Lane 5: Delta 3' splice mutant alone	25 µg
Lane 6: Target + Delta 3' ss	25 µg
Lane 7: Target+PTM29+30 (mutants)	25 µg

9

Sheet 32 of 58

## Restoration of $\beta$ -Gal Function by Double Trans-splicing

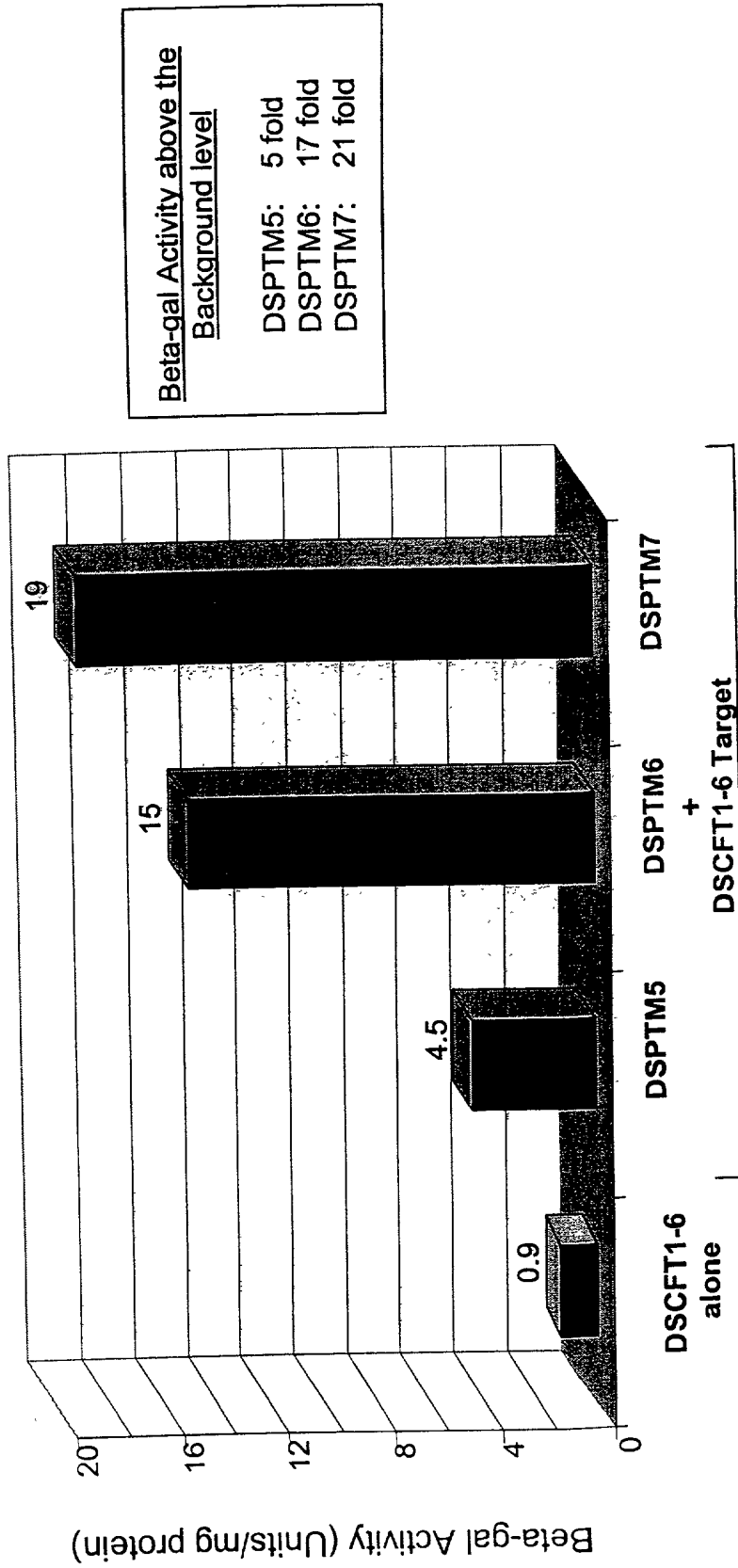


Figure 25



Sheet 33 of 58

# Restoration of $\beta$ -gal activity is due to double RNA trans-splicing events

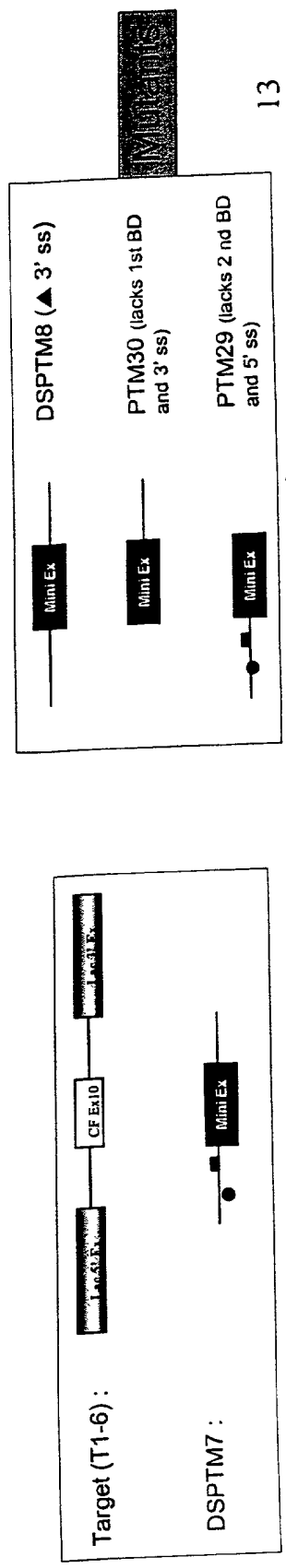
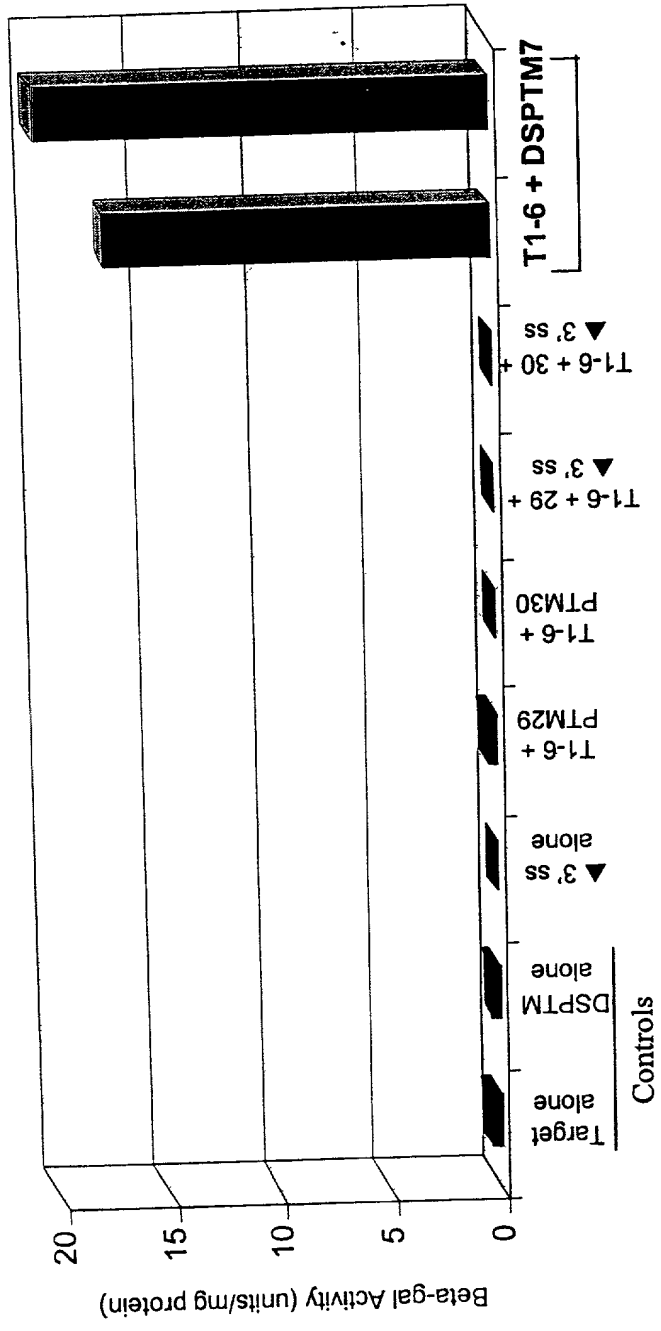


Figure 26

Sheet 34 of 58

# Double Trans-splicing: Titration of Target & PTM

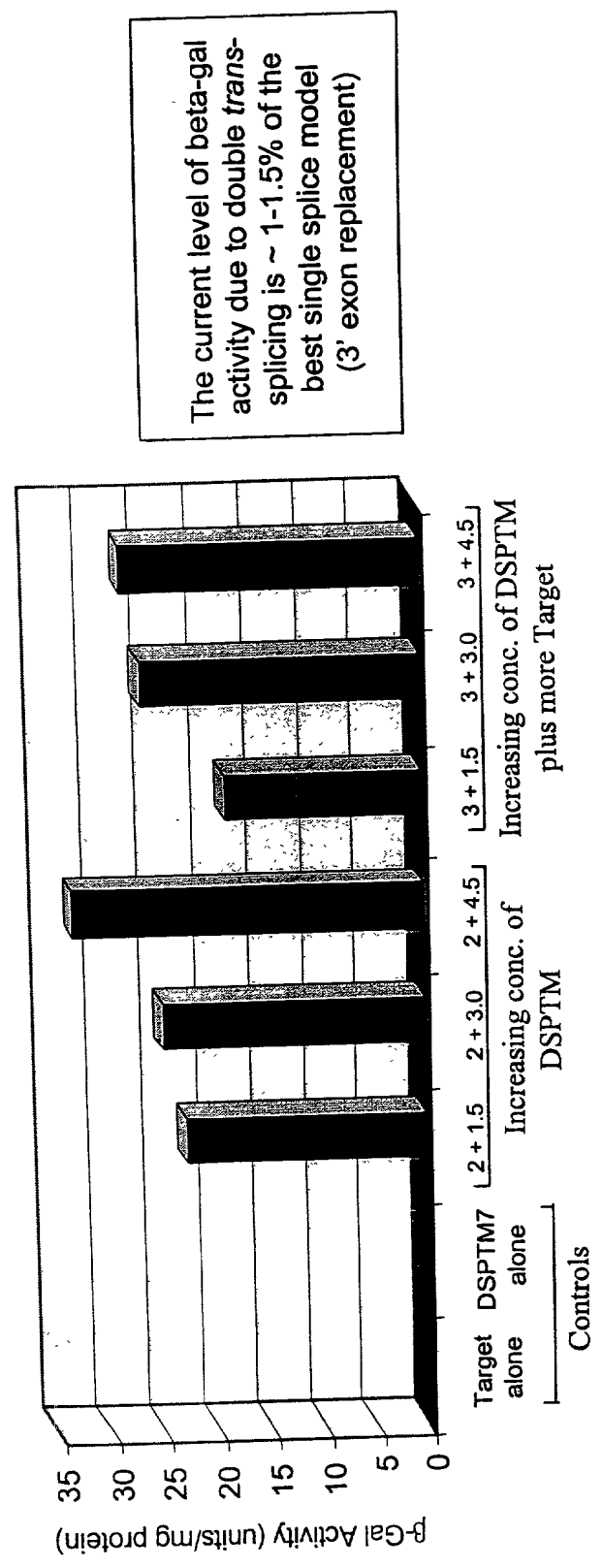
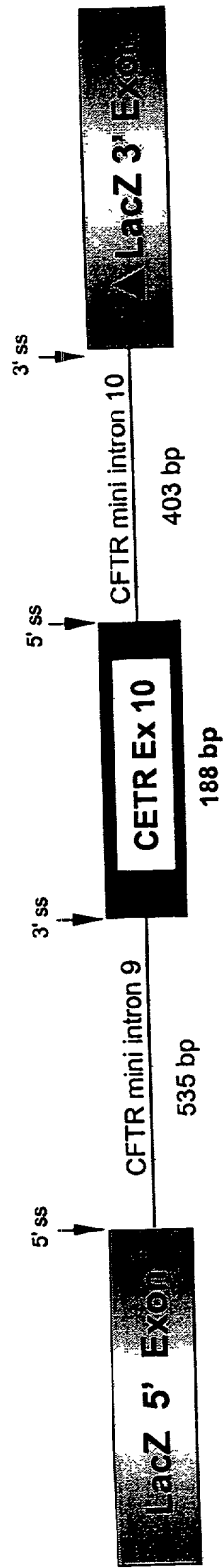


Figure 27

Sheet 35 of 58

**DSCFT1-6 (Specific Target):**



**DSHCGT1 (Non-specific Target):**

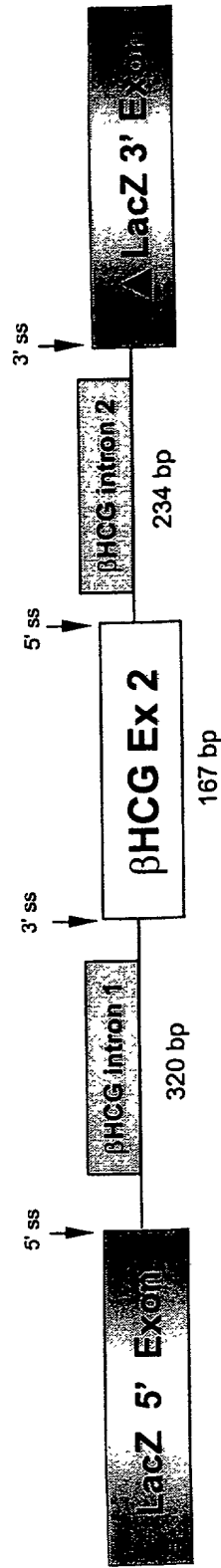


Figure 28

## Specificity of double *trans*-splicing Reaction

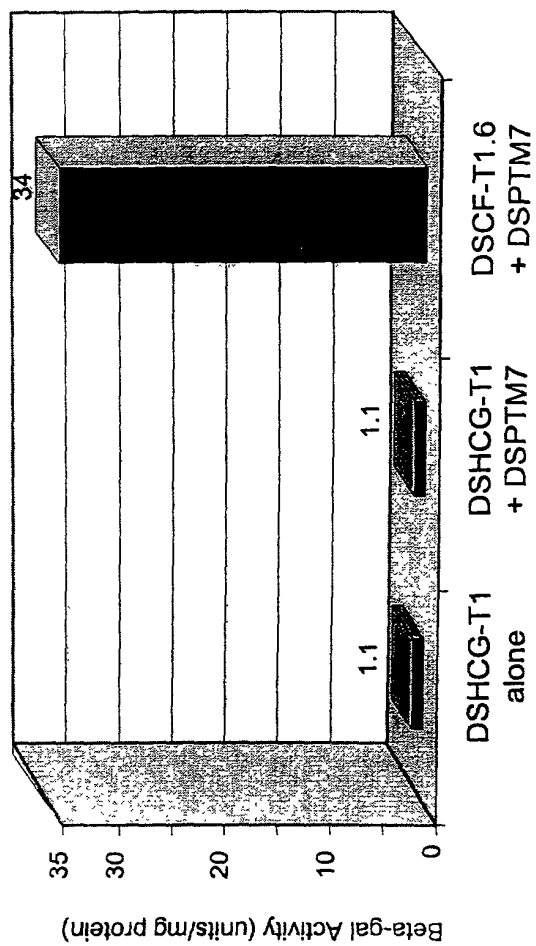
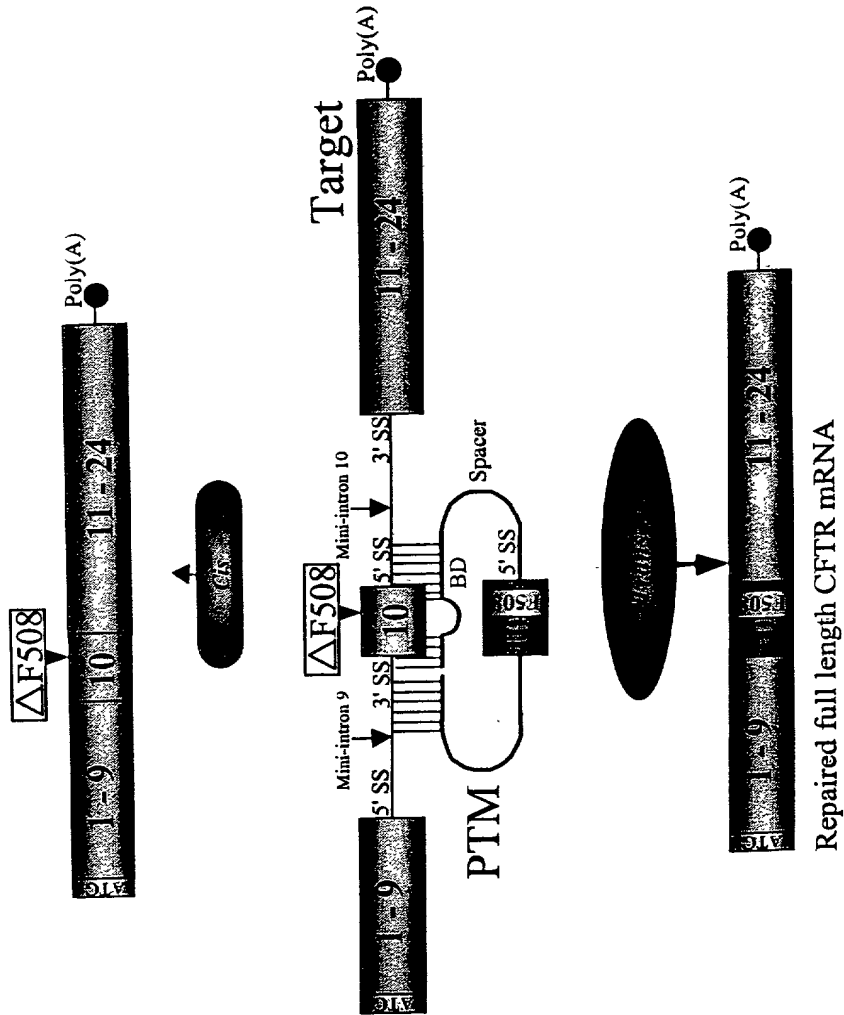


Figure 29

Sheet 36 of 58

Replacement of a Single Intron with Exon  
Schematic diagram of a PTM binding to a CFTR  $\Delta F508$  target

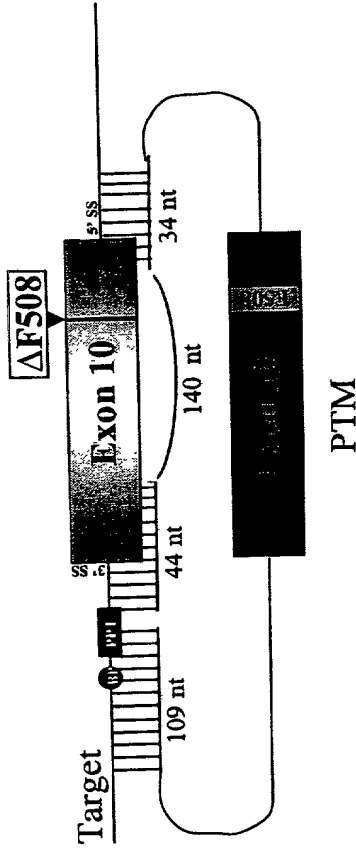


INIRONN

Figure 30

Target

PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target.



ACGAGCCTTGCTCATGATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAACATTCCCG  
GCCGCATCAGCTTTTGCAGCCCAATTTCAGTTGGATCATGCCCGGTACCATCAAGGAGAAACATAAT  
CTTCGGCGTCAGTTACGACGAGTACCGCTATCCGCTCGGTGATTAAGGCCCTGTCAGTTGGAGGAG

MCU in exon 10 of PTM  
88 of 192 (46%) bases in PTM exon 10 are not complementary to  
its binding domain (bold and underlined).

Figure 31

INTRON

About 38 of 58

Sheet 39 of 58

Target Exon 9

Sequence of a double  
trans-spliced product

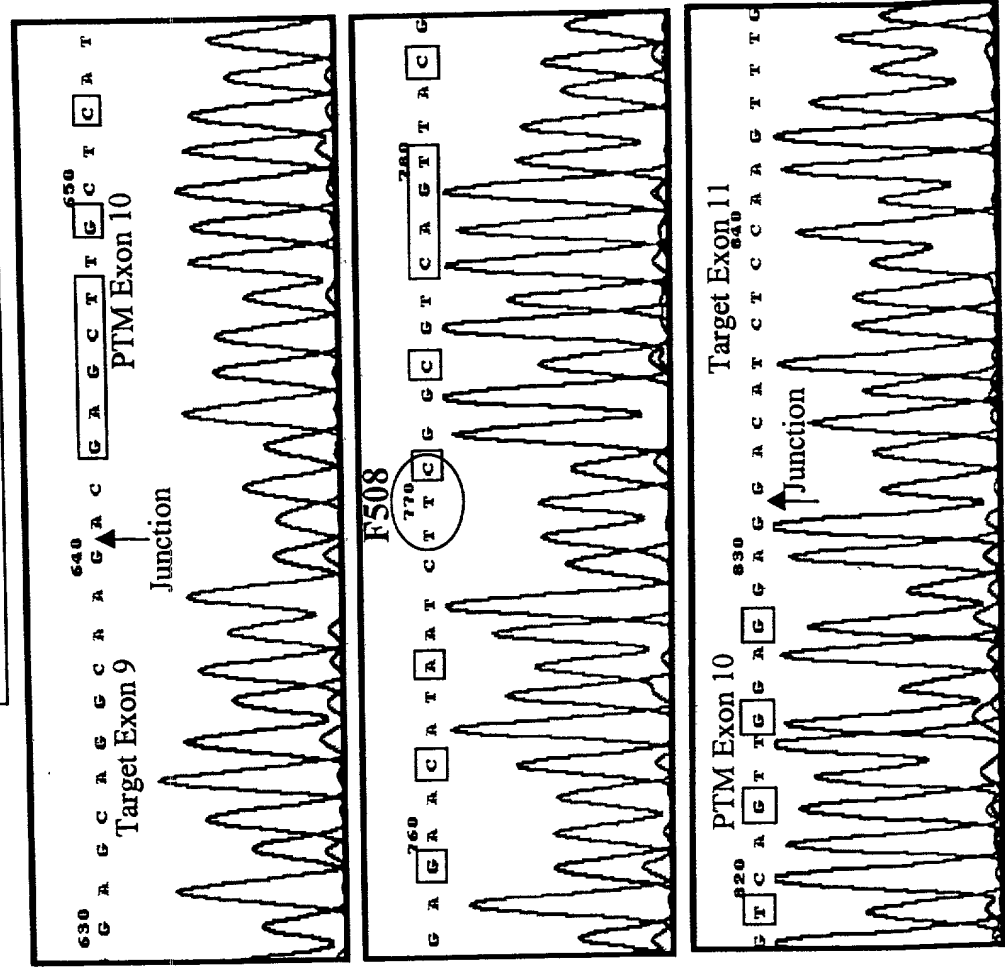


Figure 32

# CFTR Repair: 5' Exon Replacement

### Schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

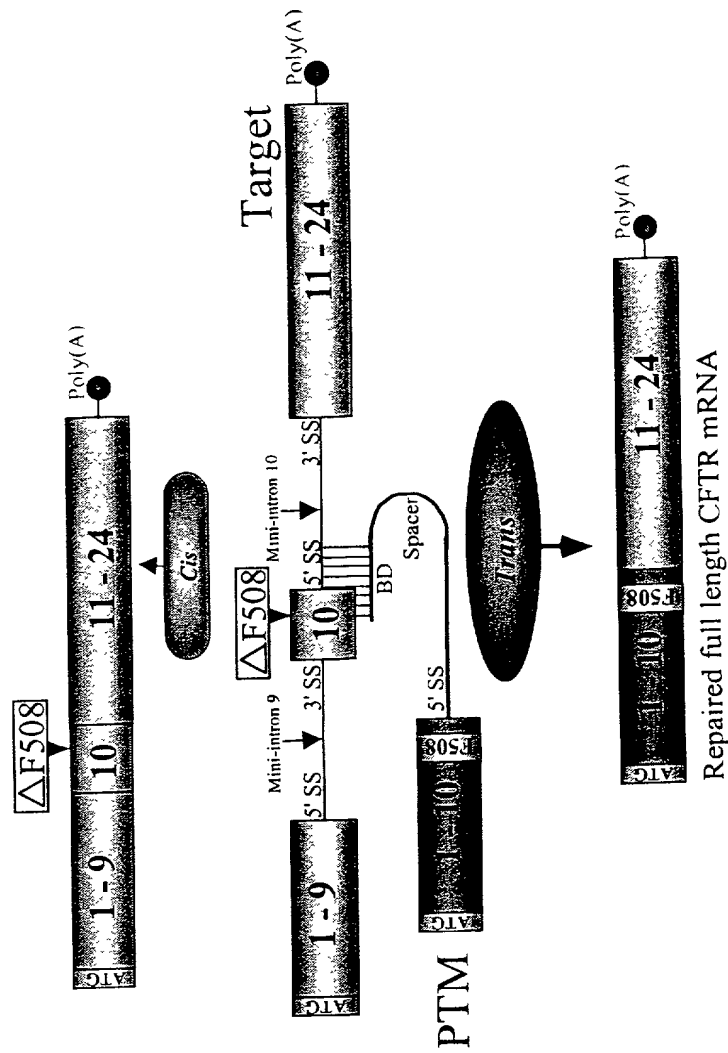
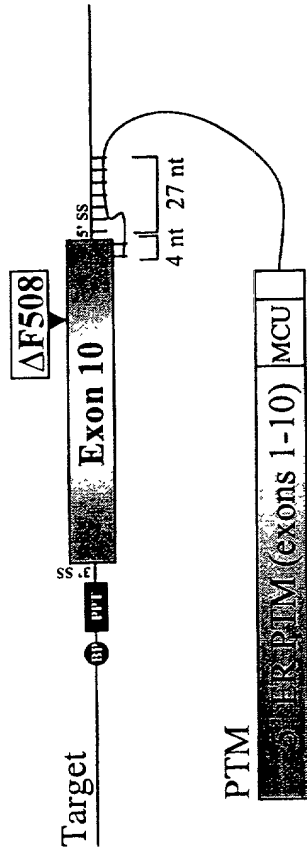


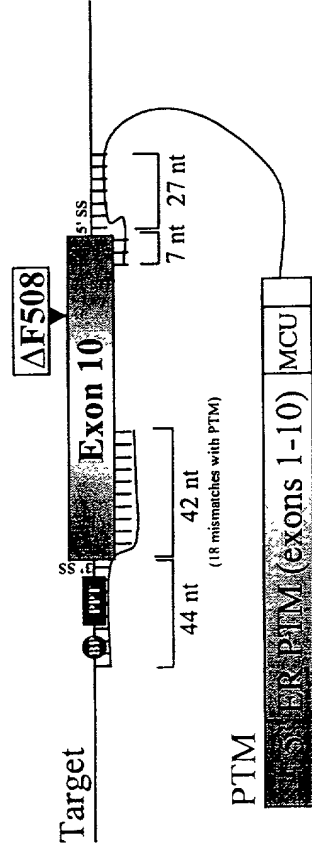
Figure 33

about 40 of 58

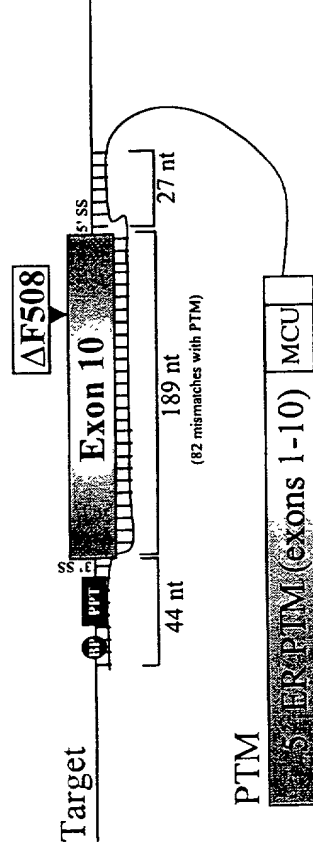




PTM with a short binding domain masking a single splice site in a mini-gene target.

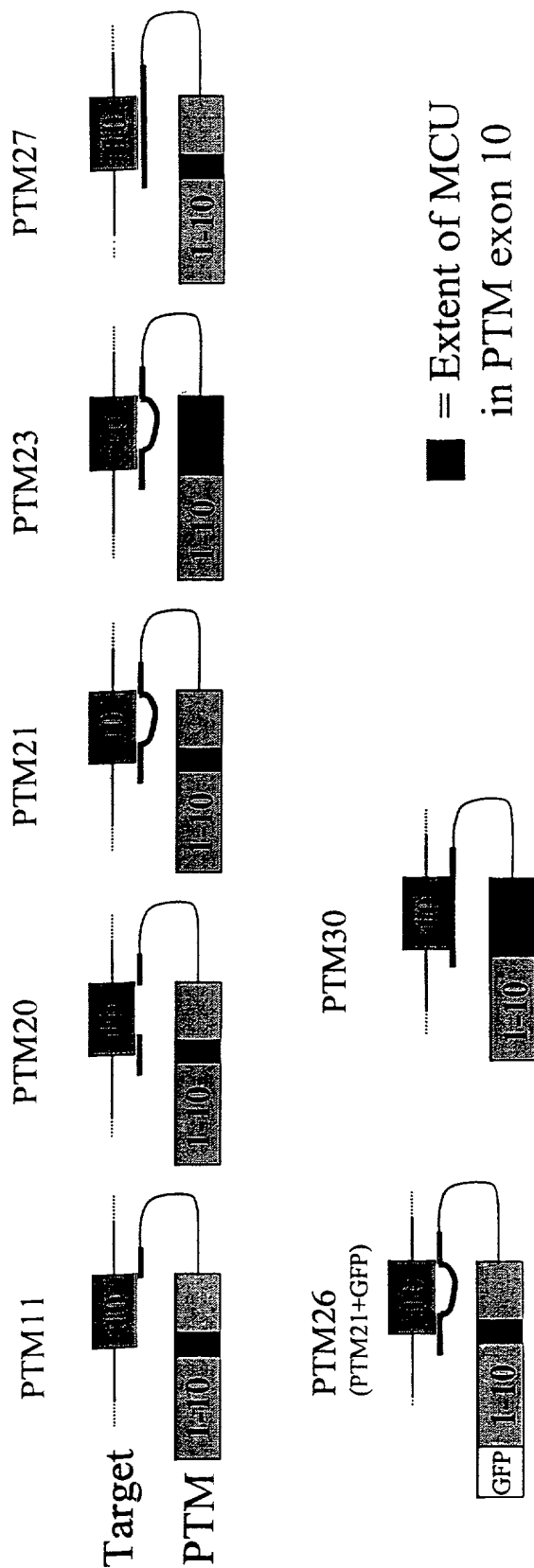


PTM with a long binding domain masking two splice sites in a mini-gene target.



PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

Figure 34



### MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGCTCATGATCATGGCGAGTTAGAACCAAGTGAAGGCAAGATCAAAACATTCCCG  
GCCGCATCAGCTTTTGCAGCCAAATTGAGTTGGATCATGCCCGGTACCATCAAGGAGAAACATAAT  
CTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAGGCCCTGTCAAGTTGGAGGAG

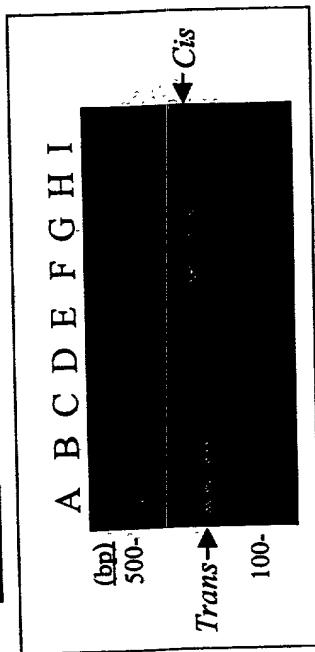
Figure 35

Sheet 43 of 58

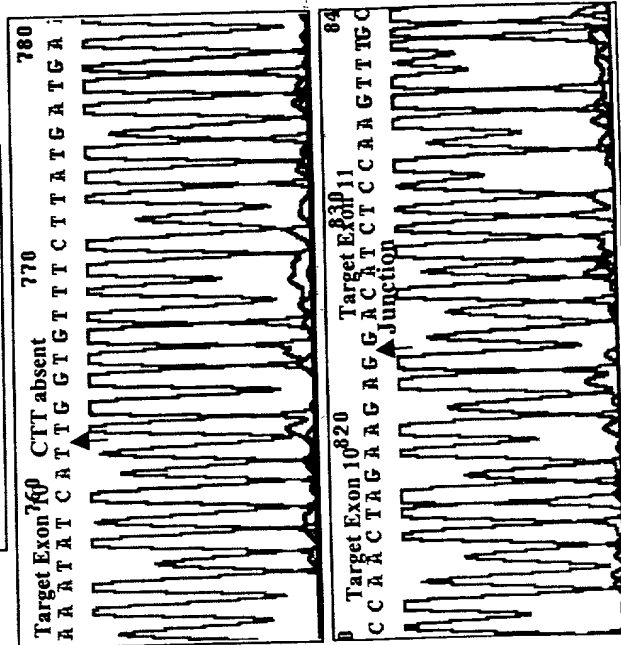
Target Exon 10 910 Target Exon 11 920

INTRON

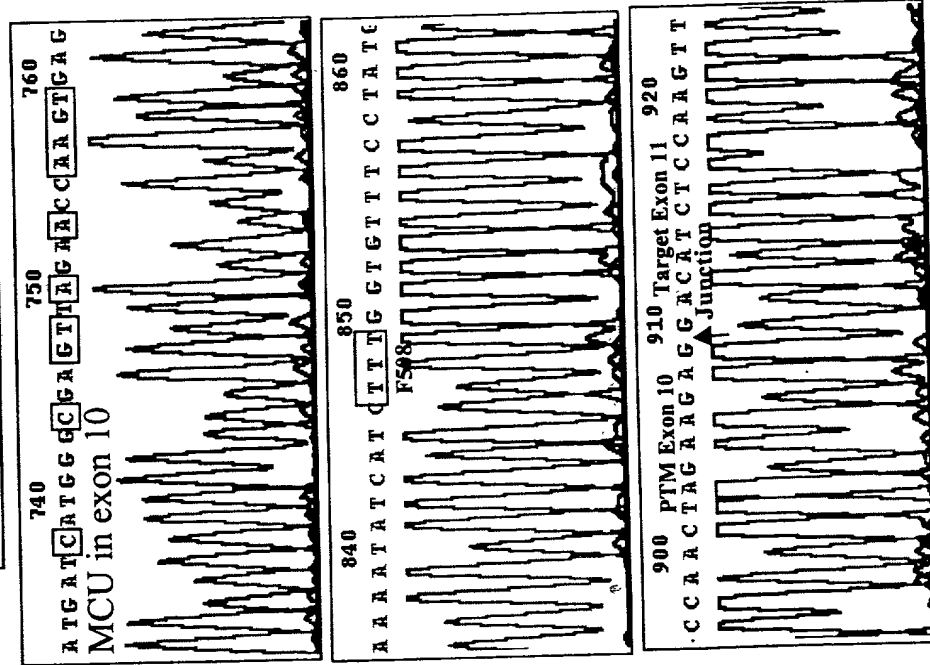
PTM Target



**A.**  
*Cis*-spliced product  
[Primers CF1 + CF111]



**B.**  
*Trans*-spliced product  
[Primers CF93 + CF111]



5  
Figure 36

A

lacZCF9m

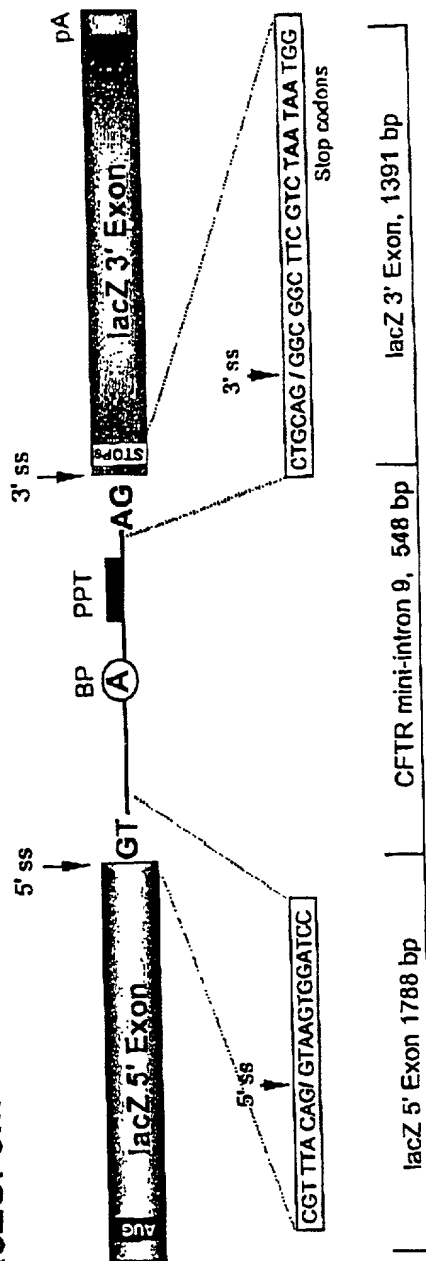


Figure 37 A

Sheet 44 of 58

Sheet 45 of 58

B

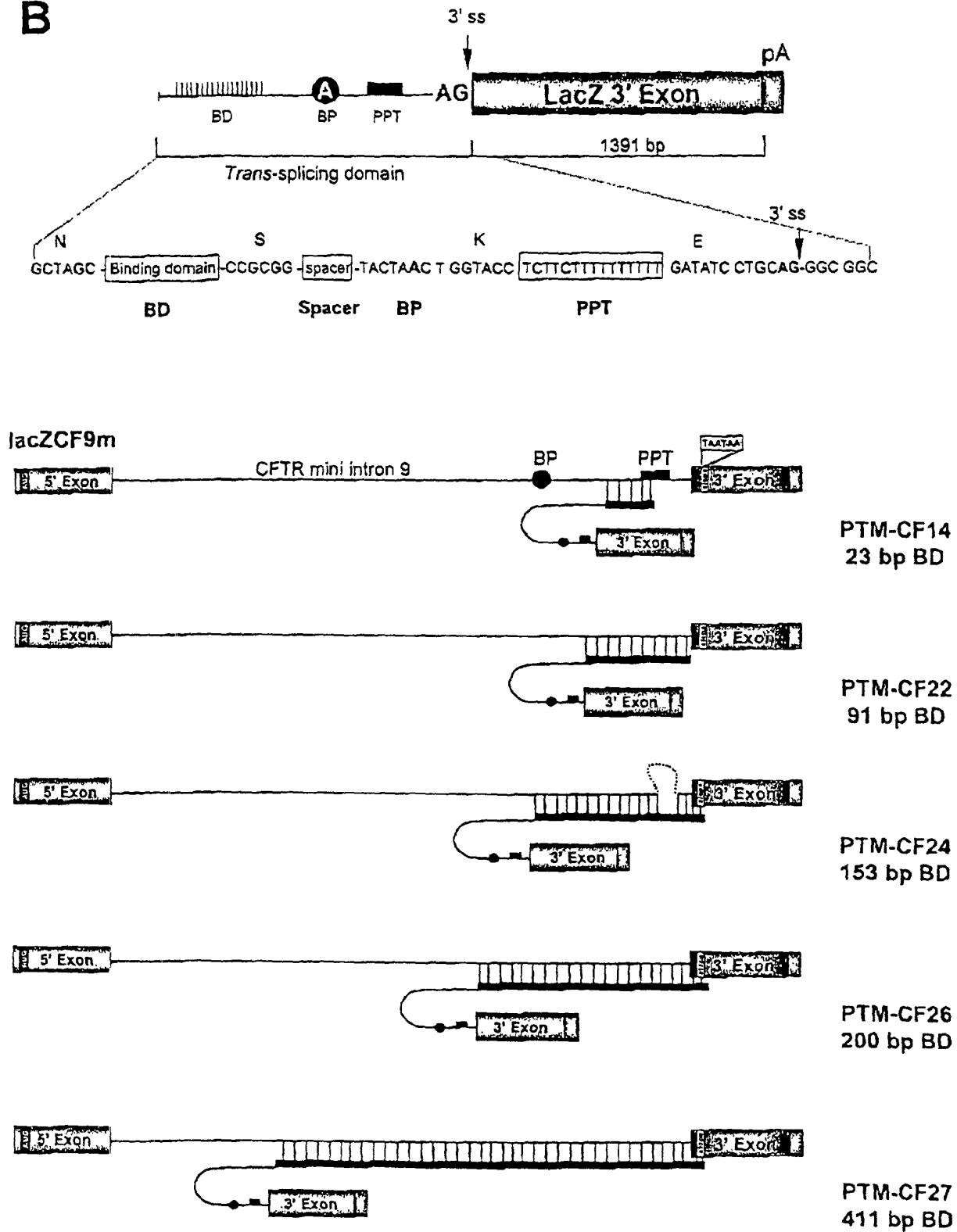


Figure 37B

Sheet 46 of 58

C

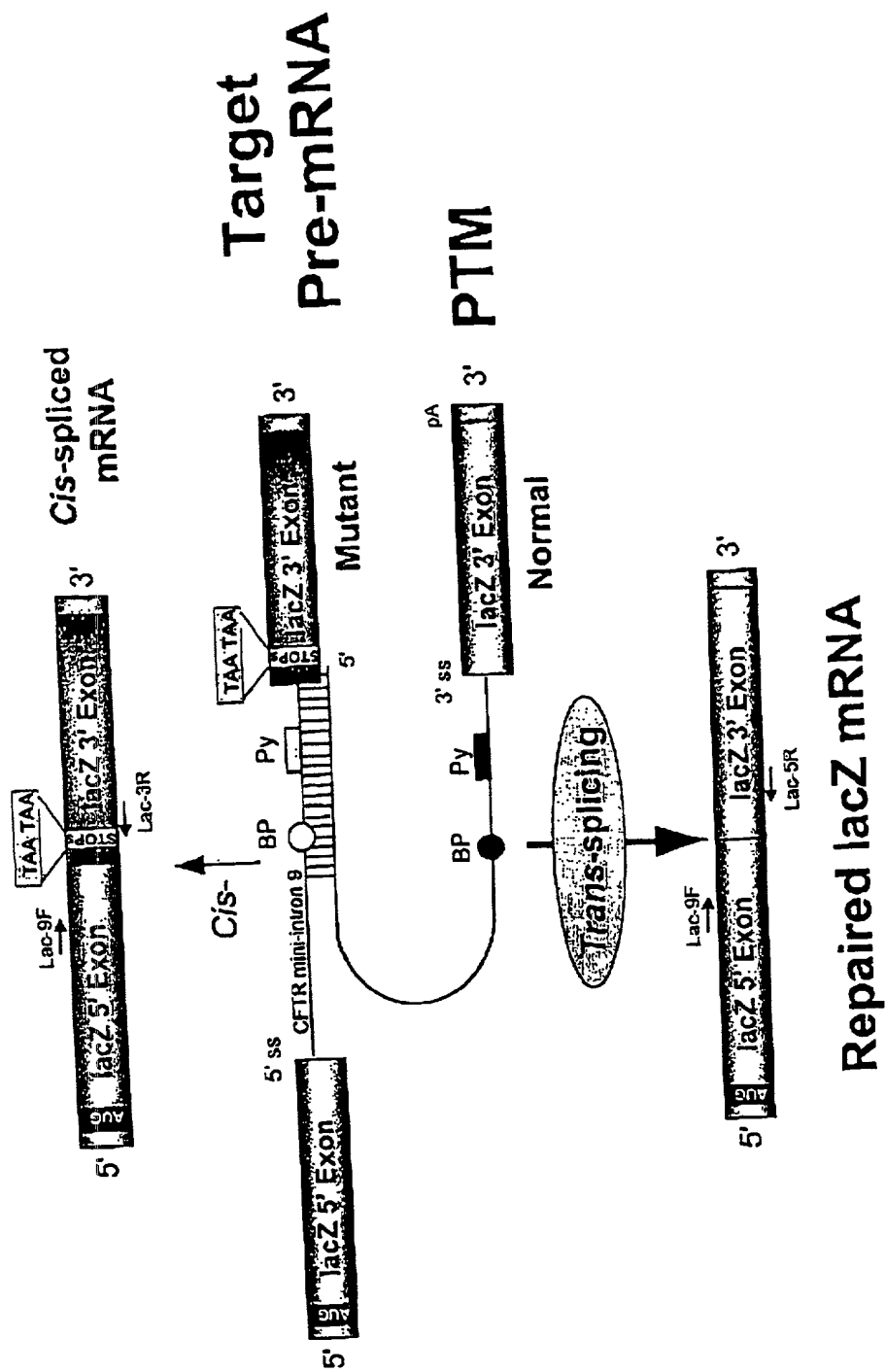


Figure 37C

Sheet 47 of 58

A

Cis-spliced  
(303 bp)

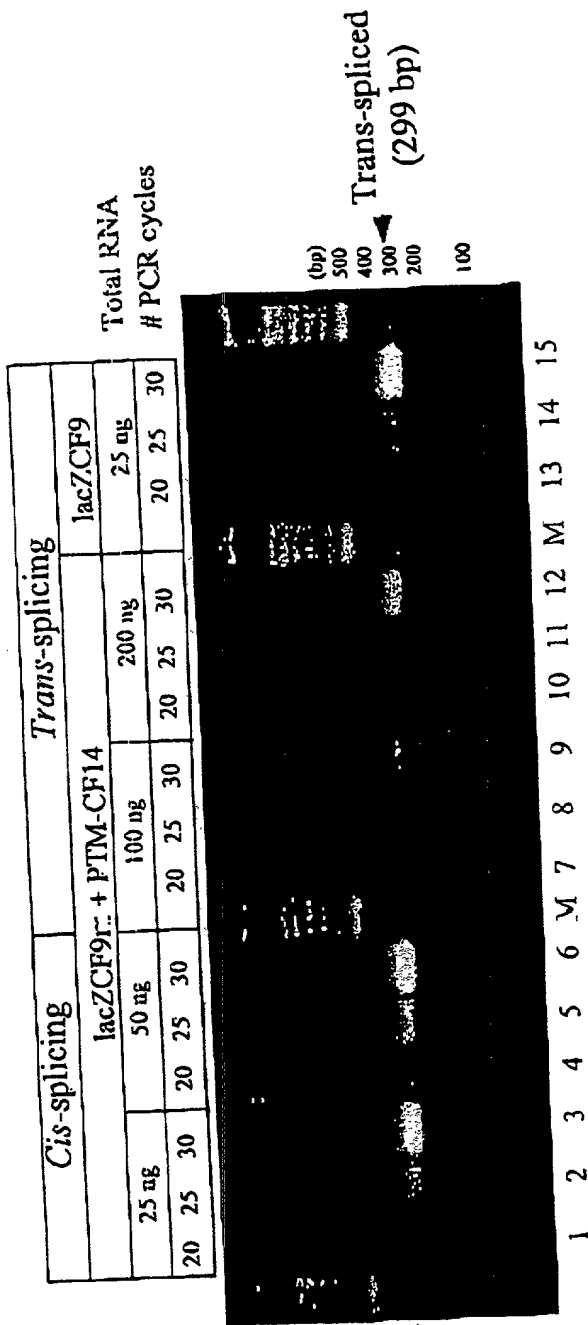
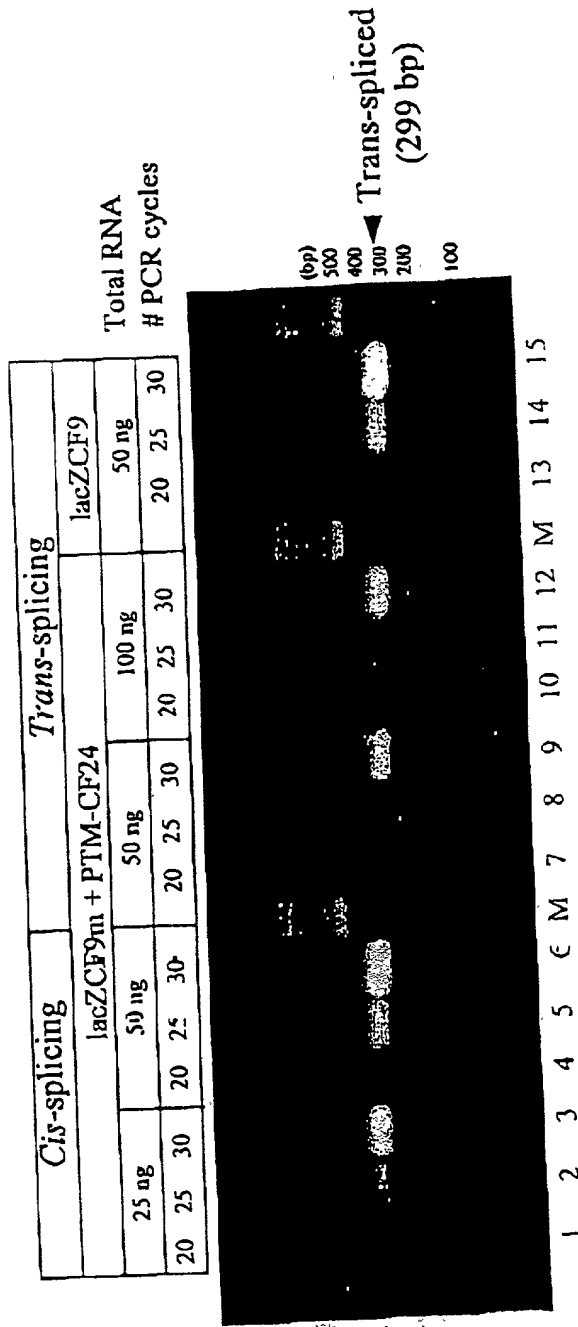


Figure 38A

Cis-spliced  
(303 bp)



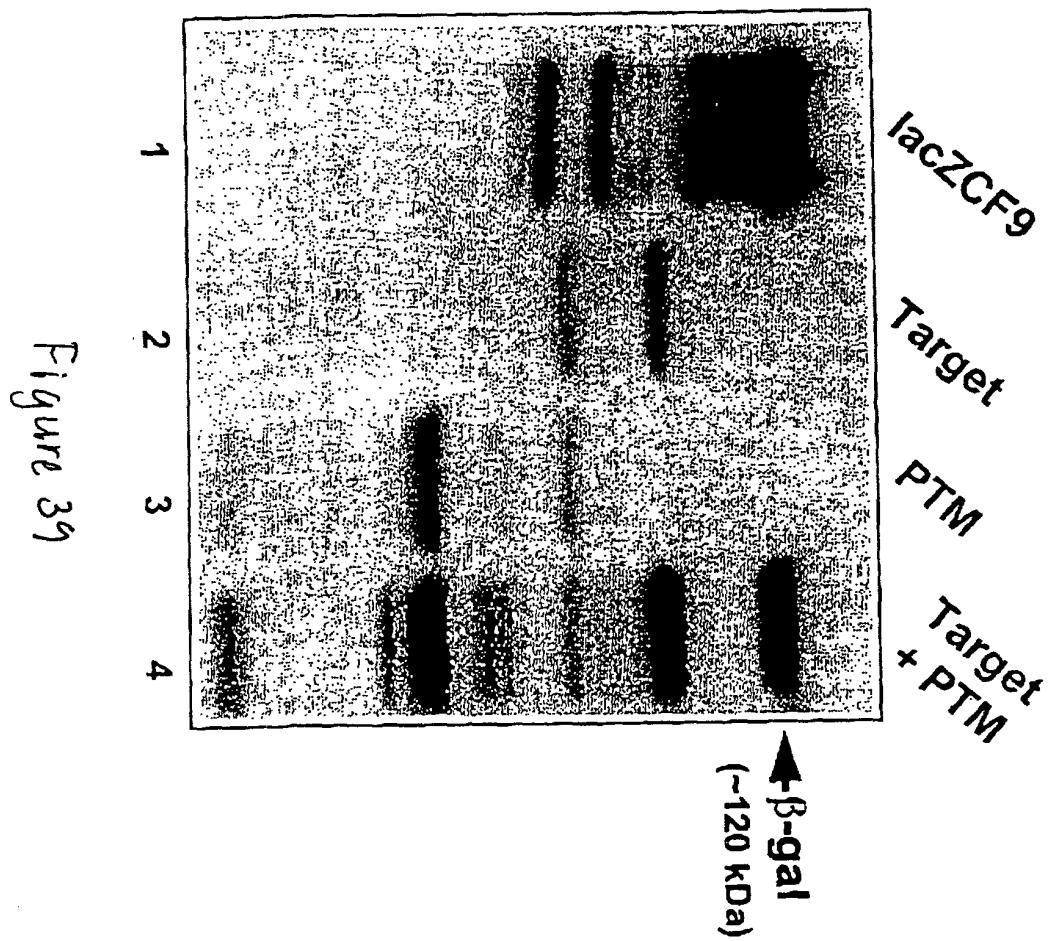
Variable	Mean	SD	Min	Max	Skewness	Kurtosis	Normality
Age	35.2	12.5	18	65	-0.1	3.2	0.98
Gender	1.2	0.4	1	2	0.1	3.1	0.99
Education	12.5	2.1	8	16	-0.2	3.3	0.97
Income	4500	1500	1000	10000	0.5	3.5	0.95
Health	2.1	0.8	1	4	-0.3	3.4	0.96
Stress	3.2	1.1	1	5	0.2	3.2	0.98
Depression	1.5	0.6	1	3	-0.1	3.1	0.99
Life Satisfaction	4.1	0.9	1	5	-0.2	3.3	0.97
Resilience	2.8	0.7	1	4	-0.1	3.2	0.98
Optimism	3.5	1.0	1	5	0.1	3.1	0.99
Gratitude	3.8	1.1	1	5	-0.1	3.2	0.98
Self-Compassion	3.0	0.8	1	4	-0.2	3.3	0.97
Emotional Regulation	3.3	0.9	1	4	-0.1	3.2	0.98
Psychological Well-being	4.0	0.8	1	5	-0.2	3.3	0.97
Life Satisfaction (Control)	4.1	0.9	1	5	-0.2	3.3	0.97
Resilience (Control)	2.8	0.7	1	4	-0.1	3.2	0.98
Optimism (Control)	3.5	1.0	1	5	0.1	3.1	0.99
Gratitude (Control)	3.8	1.1	1	5	-0.1	3.2	0.98
Self-Compassion (Control)	3.0	0.8	1	4	-0.2	3.3	0.97
Emotional Regulation (Control)	3.3	0.9	1	4	-0.1	3.2	0.98
Psychological Well-being (Control)	4.0	0.8	1	5	-0.2	3.3	0.97

PAGE. 20

DEC 28 2000 15:30



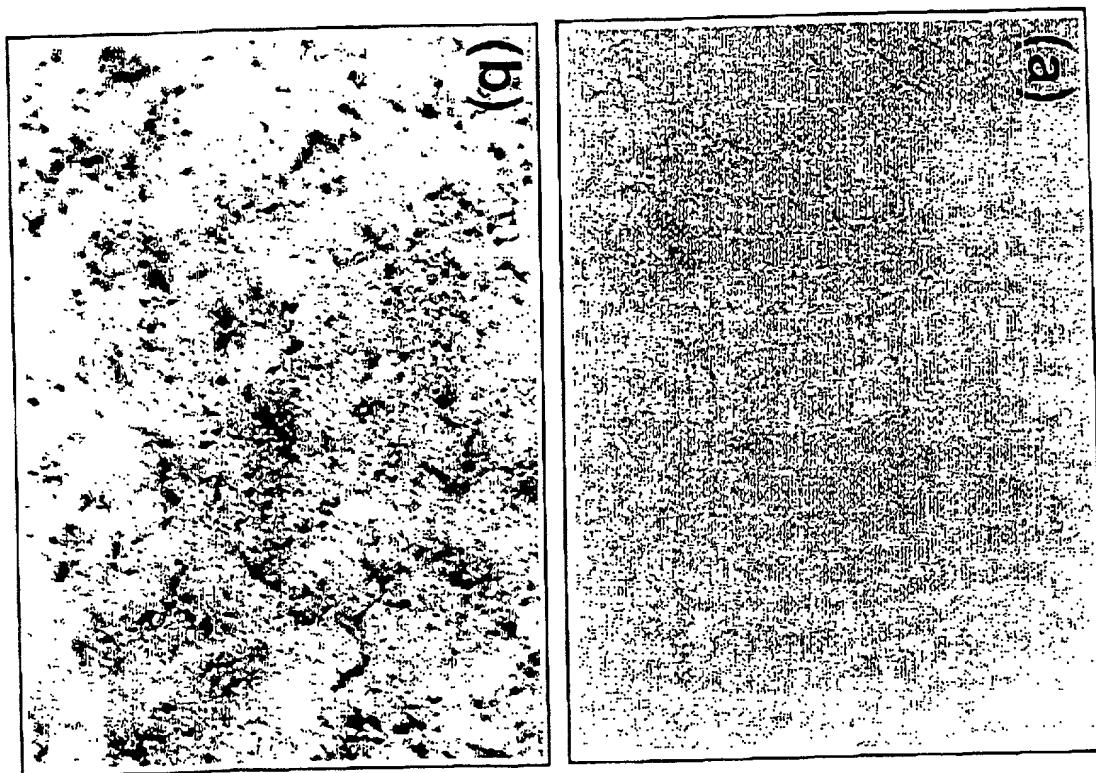




Sheet 50 of 58

Figure 40A

A



Sheet 51 of 58

B

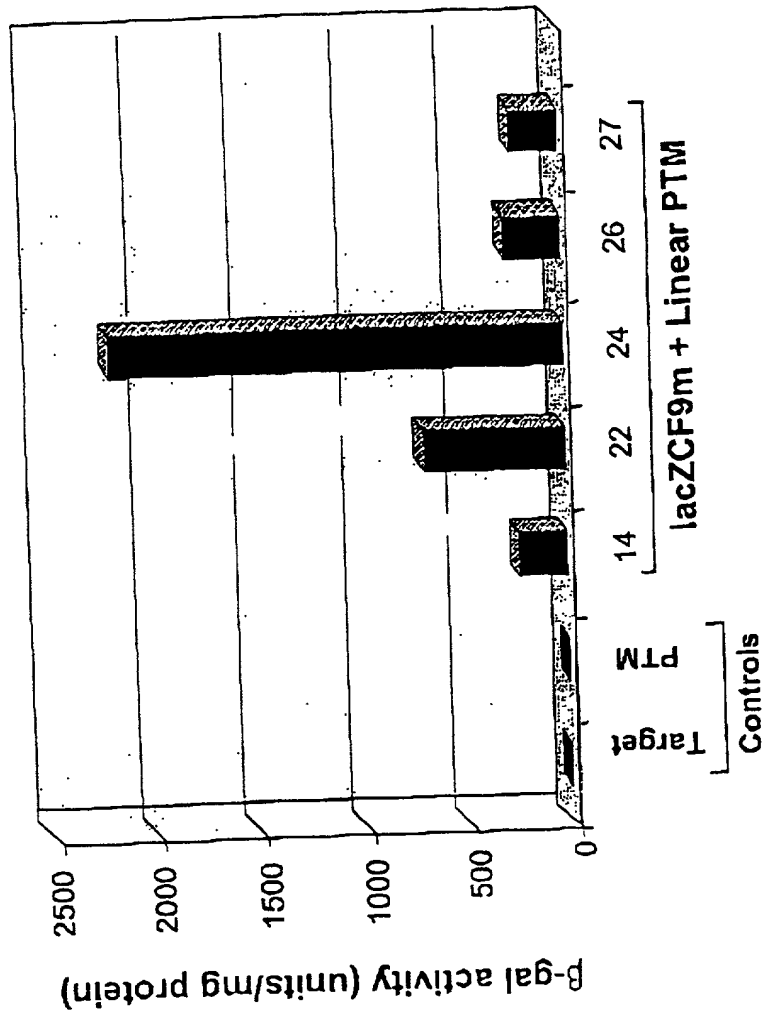


Figure 40B

Sheet 52 of 58

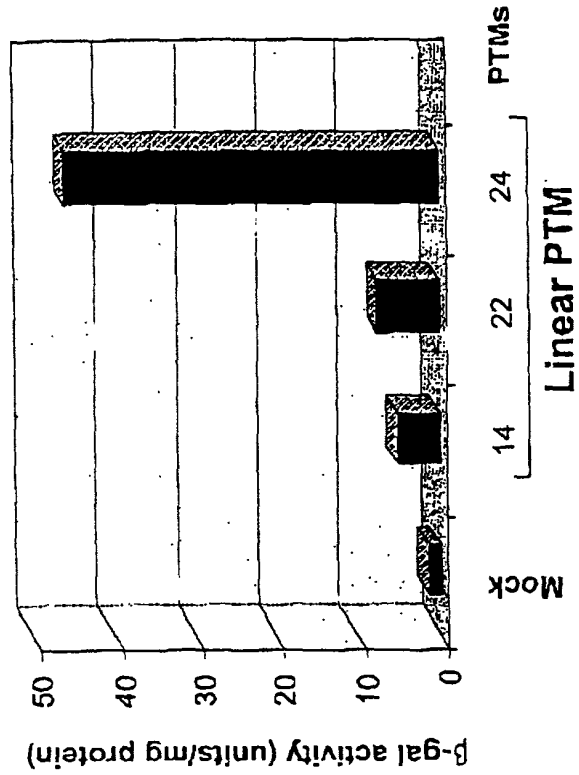


Figure 406

C

9196862129

A

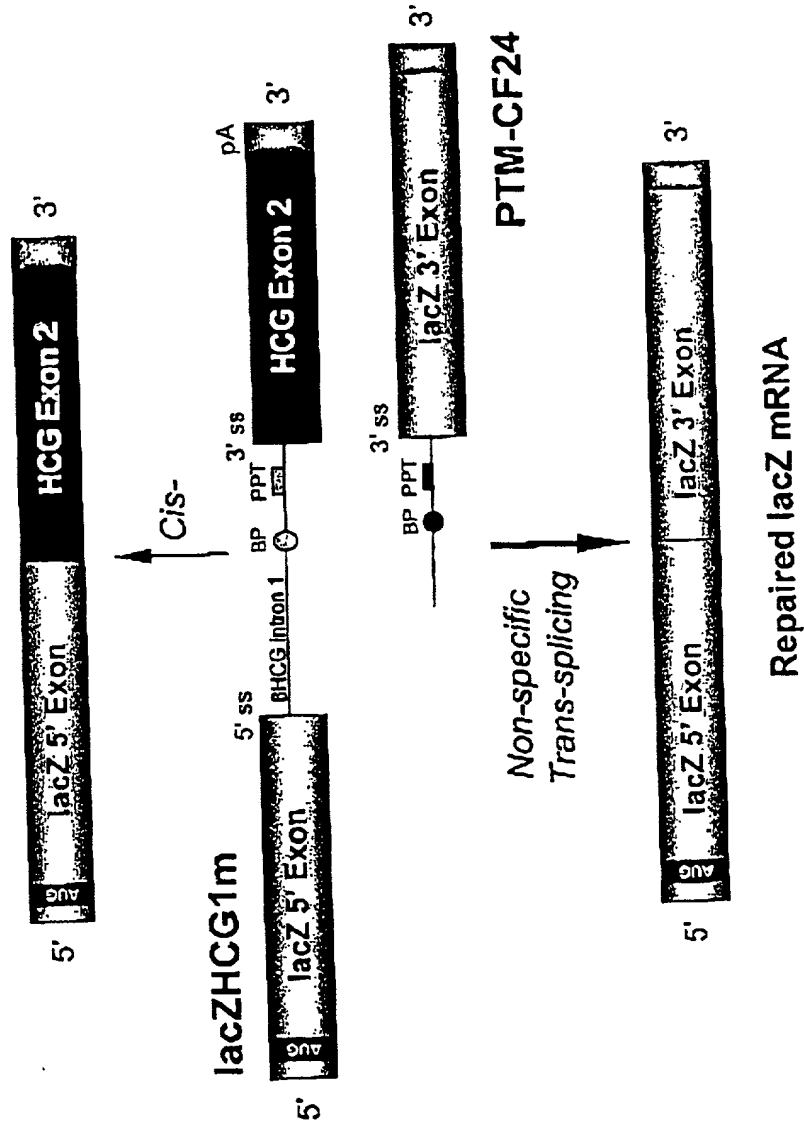


Figure 41A

Sheet 53 of 58

Sheet 54 of 58

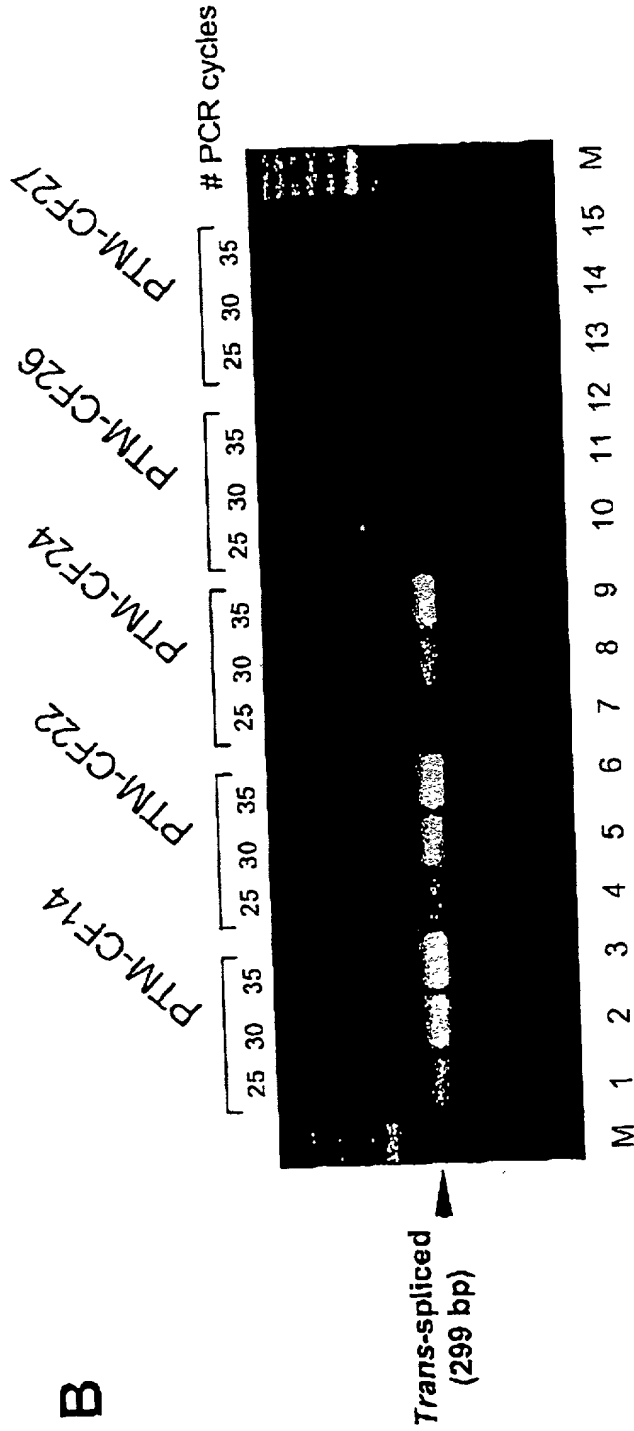


Figure 4B

Sheet 55 of 58

C

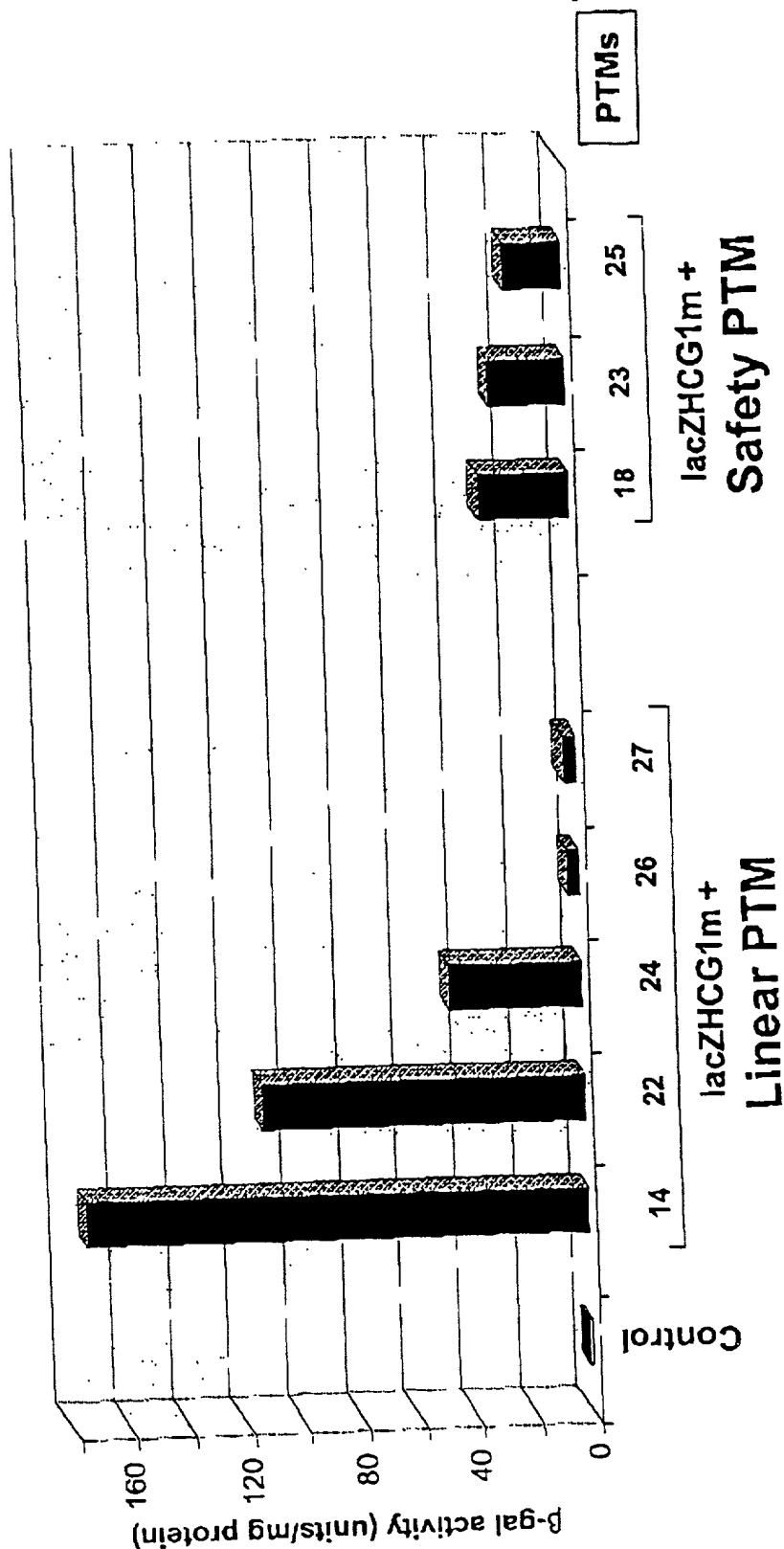


Figure 4C

Sheet 56 of 58

Exons 1-10

ATGCAGAGGTCGCCTCTGGAAAAGGCCAGCGTTGTCTCCAAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG  
GATACAGACAGCGCCTGGAATTGTGACACATATACCAAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT  
GGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAAATGCCCTTCGGCGATGTTTTTCTGG  
AGATTTATGTTCTATGGAATCTTTTTATATTTAGGGGAAGTCACCAAAGCAGTACAGCCTCTCTTACTGGGAAGAATCA  
TAGCTTCCTATGACCCGGATAACAAGGAGGAACGCTCTATCGCGATTTATCTAGGCATAGGCTTATGCCTTCTCTTTAT  
TGTGAGGACACTGCTCCTACACCCAGCCATTTTGGCCCTTCATCACATTGGAATGCAGATGAGAATAGCTATGTTTAGT  
TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTGTTAGTCTCCTTT  
CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCGTGTGGATCGCTCCTTTGCAAGTGGCACTCCT  
CATGGGGCTAATCTGGGAGTTGTTACAGGCGTCTGCCTTCTGTGGACTTGGTTTCTGATAGTCCTTGCCCTTTTTTCAG  
GCTGGGCTAGGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG  
AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAATGATTGAAAACCTTAAGACA  
AACAGAACTGAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTTCTTCTCAGGGTTCTTT  
GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCTCCGAAAAATATTCACCACCATCTCATTCT  
GCATTGTTCTGCGCATGGCGGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA  
CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATAACTTAACGACTACAGAAGTAGTGATGGAG  
AATGTAACAGCCTTCTGGGAGGAGGATTTGGGAATTATTTGAGAAAGCAAAACAAAACAATAACAATAGAAAACTT  
CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCTGAAAGATATTAATTTCAAGAT  
AGAAAGAGGACAGTTGTTGGCGGTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGGCGAG  
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGGCCGCATCAGCTTTTGCAGCCAATTCAGTTGGATCATGCCCGGTA  
CCATCAAGGAGAACATAATCTTCGGCGTCAGTTACGACGAGTACCGCTATCGCTCGGTGATTAAAGGCCTGTCAGTTGGA  
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAAGATCCACCGG  
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG  
GAAACACCAATGATATTTTCTTTAATGGTGCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGT  
GCTTAATTTTACCCTCTGAATTTCTCCATTTCTCCATAATCATCATTACAACCTGAACCTCTGGAATAAAACCCATCATT  
ATTAACCTATTATCAAATCAGCT

Figure 42



153 bp PTM24 Binding Domain:

Nhe I

153 bp BD underlined

GCTAGC - AATAAT GACGAAGCCGCCCTCAGCTCAGGATTCACCTTGCCCTCCAATTATCATCCCTAAGCAGAGTGTATA

TTCTTATTGTAAAGATTCTATTAACTCATTTGATTCAAATAATTTAAATACTTCTGTTTCACCTACTCTGCTATGC

Sac II

AC - **CCGCGG**

Figure 43A

Sheet 58 of 58

Trans-splicing domain

AATAATGACGAAGCCGCCCTCAGCTCAGGATTCAGTTGCCCTCCAATTATCATCCTAAGCAGAAGTGATATTTCTTA  
TTTGTAAGATTCTATTAACTCATTGATTCAAAATATTTAAATACTTCCTGTTTCACCTACTCTGCTATGCACCCGC  
GGAACATTATTATAACGTTGCTCGAATACTAAGTGGTACCTCTTCTTTTTTTTTTGATATCCTGCAG

Exons 10-24

ACTTCACCTCTAATGATGATTATGGGAGAAGTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGAAGAATTTCACTCT  
GTTCTCAGTTTTCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTCCTATGATGAATATAGATA  
CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTGCAGAGAAAGACAATATAGTTCTTGGAGAA  
GGTGAATCACTAGTGGAGGTCAACGAGCAAGAATTTCTTAGCAAGAGCAGTATACAAAGATGCTGATTGTGATT  
TATTAGACTCTCCTTTTGATACCTAGATGTTTAAACAGAAAAAGAAATATTTGAAAGCTGTGTCTGTAAACTGATGGC  
TAACAAAACCTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAGGT  
AGCAGCTATTTTATGGGACATTTTCAGAACTCCAAATCTACAGCCAGACTTTAGCTCAAACTCATGGGATGTGATT  
CTTTTCGACCAATTTAGTGCAGAAAGAGAAATTCATCTTAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT  
TCCTGTCTCCTGGACAGAAACAAAAACAATCTTTAAACAGACTGGAGAGTTGGGGAAAAAGGAAGAATTTCTATT  
CTCAATCCAATCAACTCTATACGAAAATTTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT  
CTGATGAGCCTTTAGAGAGAAGGCTGTCTTAGTACCAGATCTGAGCAGGGAGAGGCGATACTGCCTCGCATCAGCGT  
GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGAGTCTGTCTGAACCTGATGACACACTCAGTTAACCAAGGT  
CAGAACATTCACCGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCCCTCAGGCAAACTGACTGAACTGGATA  
TATATTTCAAGAAGGTTATCTCAAGAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAAGGAGTGCTT  
TTTTGATGATATGGAGAGCATACCAGCAGTGACTACATGGAACACATACCTTCGATATATTACTGTCCACAAGAGCTTA  
ATTTTTGTGCTAATTTGGTGCTTAGTAATTTTCTGGCAGAGGTGGCTGCTTCTTTGGTGTGTCTGTGGCTCCTTGGAA  
ACACTCCTCTTCAAGACAAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTC  
GTATTATGTGTTTTACATTTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCACTGGTG  
CATACTCTAATCACAGTGTGAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCCCTCA  
ACACGTTTGAAGCAGGTGGGATTCTTAATAGATTCTCCAAAGATATAGCAATTTTGGATGACCTTCTGCCTCTTACCAT  
ATTTGACTTCATCCAGTTGTTATTAATTTGTGATTGGAGCTATAGCAGTTGTGCGAGTTTACAACCCTACATCTTTGTT  
GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCTCCAAACCTCACAGCAACTCAAACAAGTGG  
AATCTGAAGGCAGGAGTCCAATTTCACTCATCTTGTTACAAGCTTAAAGGACTATGGACACTTCGTGCCTTCGGACG  
GCAGCCTTACTTTGAAACTCTGTTCACAAAGCTCTGAATTTACATACTGCCAACTGGTTCTTGTACCTGTCAACACTG  
CGCTGGTTTCCAAATGAGAATAGAAATGATTTTGTCACTTCTTTCATTGCTGTACCTTCATTTCCATTTTAAACAAG  
GAGAAGGAGAAGGAAGAGTTGGTATTATCCTGACTTTAGCCATGAATATCATGAGTACATTGACAGTGGGCTGTAAACTC  
CAGCATAGATGTGGATAGCTTGATGCGATCTGTGAGCCGAGTCTTTAAGTTTATTGACATGCCAACAGAAGGTAAACCT  
ACCAAGTCAACCAACCATACAAGAATGGCCAACTCTCGAAAGTTATGATTATTGAGAATTCACAGTGAAGAAAGATG  
ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCACAGCAAAATACACAGAAGGTGGAAATGCCATATTAGA  
GAACATTTCTCTCAATAAGTCTGGCCAGAGGGTGGGCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTA  
TCAGCTTTTTTGGAGTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGTCTTGGGATTCAATAACTTTGCAAC  
AGTGGAGGAAAGCCTTTGGAGTGATACCACAGAAAGTATTTATTTTTCTGGAACATTTAGAAAAAACTTGGATCCCTA  
TGAACAGTGGAGTGATCAAGAAATATGGAAGTTGCAGATGAGGTTGGGCTCAGATCTGTGATAGAACAGTTTCTGGG  
AAGCTTGACTTTGTCTTGTGGATGGGGGCTGTGTCTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG  
TTCTCAGTAAGGCGAAGATCTTGCTGCTTGATGAACCCAGTGCTCATTTGGATCCAGTAACATACCAATAATTAGAAG  
AACTCTAAAAACAAGCATTTGCTGATTGCACAGTAATCTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA  
TTTTTGGTCATAGAAGAGAACAAAGTGCAGCAGTACGATTCATCCAGAAACTGCTGAACGAGAGGAGCCCTTCCGGC  
AAGCCATCAGCCCCCTCCGACAGGGTGAAGCTCTTTCCCCACCGAACTCAAGCAAGTGCAAGTCTAAGCCCCAGATTGC  
Histidine tag Stop  
TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATCATTAG

Figure 43B